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Embeli Ribes Burm

Halicacabum Leaves Extracts

Highlights

Protein and Lipid Glycation

Medicinal Property of Camphor

Discovering Thoughts, Inventing Future

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Contents of the Volume

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Chemistry, Pharmacology and Medicinal Property of Camphor (Cinnamomum Camphora) Traditional Remedy with the History of Treating Several Diseases

By Rafie Hamidpour, Soheila Hamidpour, Mohsen Hamidpour & Mina Shahlari

Abstract- Camphor (Cinnamomum camphora) has been known as a Traditional Remedy and Natural Medicine from ancient times for the cure or relief of some illnesses and their symptoms. Cinnamomum Camphora is considered important for drug development, because there are many reports of their pharmacological activities throughout the world, especially in Middle East, China and India. The purpose of this study is to gain knowledge of the long history, wide variety and extensive applications of camphor both in traditional and modern medicine. Camphor (Cinnamomum camphora) which is obtained from the wood of camphor tree, has been used for centuries and throughout the world as a remedy for treating variety of symptoms such as inflammation, ingestion, infection, congestion, pain, irritation, etc.

Keywords: *cinnamomum camphora, application, treatment, cancer.*

GJMR-B Classification : *NLMC Code: WB 330, QV 752*

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Rafie Hamidpour [α], Soheila Hamidpour [σ], Mohsen Hamidpour [ρ] & Mina Shahlari [ω]

Abstract- Camphor (Cinnamomum camphora) has been known as a Traditional Remedy and Natural Medicine from ancient times for the cure or relief of some illnesses and their symptoms. Cinnamomum Camphora is considered important for drug development, because there are many reports of their pharmacological activities throughout the world, especially in Middle East, China and India. The purpose of this study is to gain knowledge of the long history, wide variety and extensive applications of camphor both in traditional and modern medicine. Camphor (Cinnamomum camphora) which is obtained from the wood of camphor tree, has been used for centuries and throughout the world as a remedy for treating variety of symptoms such as inflammation, ingestion, infection, congestion, pain, irritation, etc. The studies have shown that some of the components of Cinnamomum camphora have suppressive and anti-mutagenic effect in number of human cancer cells without harming the healthy cells. In this paper our focus is on the use of camphor, as a remedy for daily minor problems as well as gathering some information about the new applications of this traditional medicine to treat or prevent some other serious, life threatening diseases like cancer, diabetes, or perhaps getting the attention of researchers for conducting more studies on the patients with memory and brain disorders as well.

Keywords: cinnamomum camphora, application, treatment, cancer.

I. Introduction

Camphor is a white, crystalline substance with a strong odor and pungent taste, derived from the wood of Camphor laurel (Cinnamomum camphora) and other related trees of laurel family. Camphor tree is native to China, India, Mongolia, Japan and Taiwan and a variety of this fragrant evergreen tree is grown in Southern United States; especially in Florida [1, 2]. Camphor is obtained through steam distillation, purification and sublimation of wood, twigs and bark of the tree [3]. There are many pharmaceutical applications for camphor such as: topical analgesic, antiseptic, antispasmodic, anti-pruritc, anti-inflammatory, anti-infective, rubefacient, contraceptive, mild expectorant, nasal decongestant, cough suppressant, etc. [3-5]. Camphor is easily absorbed through the skin, and also can be administrated by injection, inhalation and ingestion [3, 6].

Camphor (Cinnamomum camphora) has several chemical varieties, each with different essential oil compositions [1]. The leaf of Cinnamomum camphora contains camphor, as the main component along with 1-8 cineol, linalool, eugenol, limonene, safrole, α-pinene, β-pinene, β-myrecene, α-humulene, p-cymene, nerolidol, borneol, camphene and some other components [1, 5, 7, 8].

II. Chemical Constituents

Camphor laurel contains volatile chemical compounds in all plant parts, and the wood and leaves are steam distilled for the essential oils. Camphor laurel has six different chemical variants called chemo types, which are camphor, linalool, 1,8-cineole, nerolidol, safrole, or borneol. In China field workers avoid mixing chemo types when harvesting by their odors [26, 27]. The cineole fraction of camphor laurel is used in China to manufacture fake "Eucalyptus oil" [28].

The chemical variants (or chemo types) seem dependent upon the country of origin of the tree. The tree is native to China, Japan, and Taiwan. It has been introduced to the other countries where it has been found, and the chemical variants are identifiable by country i.e., Cinnamomum camphora grown in Taiwan and Japan, (often commonly called "Ho Wood") is normally very high in Linalool, often between 80 and 85%. In India and Sri Lanka the high camphor variety/chemo type remains dominant. The Cinnamomum camphora grown in Madagascar, on the other hand, is high in 1, 8 Cineole (averaging between 40 and 50%). The essential oil from the Madagascar trees is commercially known as Ravintsara [29].

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III. Applications

Camphor is a natural product with many applications in traditional and modern medicines. Traditionally camphor has been used as a cold remedy for the relief of chest congestion, and the treatment of inflammation related diseases such as rheumatism, sprains, bronchitis, asthma, ingestion and muscle pain [9]. Camphor is usually prepared as a balm, oil or cream to relieve the pain and inflammation in joints and muscles. Camphor oil (20% camphor in cotton seed oil), when applied on the skin produces the feeling of coolness which is related to the stimulation of nerve endings sensitive to cold. Camphor activates some of TRP (transient receptor potential) channels like TRPV1, TRPV3, TRPM8 and inhibits TRPA1, causing the warm sensation, excitation and desensitization of sensory nerves, relieving the pain, itch and irritation in applied area [4, 10, 11, 12].

There are many reports which prove that the use of camphor, solely or in combination with other treatments can be very effective for treating and preventing some serious diseases. A cancer study shows that the use of camphor odor as a conditioning agent for the cancer cells of YC8 lymphoma in mice could have a suppressive effect on the growth of YC8 tumor, when it is combined with immunotherapy treatment [13]. Camphor also can be potential radio-sensitizing agent in radiotherapy. Treatment with camphor prior to a radiation showed the reduced growth of tumor volume [3].

A camphor based drug called 714-X, was developed by a Canadian researcher more than forty years ago and it is believed by some institutions, to be effective on the treatment of some patients with cancer, especially breast and prostate cancer [14]. Padma 28 is another multi compound herbal preparation, based on camphor formulas which have shown to be effective against chronic inflammatory diseases. The result of a study indicates that Padma 28 has the ability to suppress the development of autoimmune diabetes in female non-obese diabetic (NOD) mice which could be an experimental model for type1diabetes mellitus in human [15].

There are a number of applications known for different parts of Cinnamomum camphora tree. The study of Cinnamomum camphora leaves extract (CLE) has shown the protective effects of this extract against DNA damage and biochemical changes in mice caused by Atrazine (AT) which is one of the commonly used grasses and weed herbicides [9]. The widespread usage of AT has caused contamination in the environment, resulting in genotoxicity and biochemical disturbances in animals and human cells. In this experiment, all the tested tissues which were treated with CLE showed a significant and time dependant decrease in chromosomal abnormalities and DNA damage [9]. Two ribosome inactivating proteins (RIPs), cinnamomin and camphorin are found; studies have shown their inhibitory effect on the cultured carcinoma cells [16]. In addition, cinnamomin has shown to have inhibitory effect on the growth of solid melanoma in the skin of the nude mouse [16].The application of RIPs can be very significant in drug development and crop-plant technology due to their toxicity against viruses, tumor cells, insects and plant fungal pathogens [17].

In recent years, the finding of carbon nanotubes (CNT) which are made of very light and strong fibers of one atom-thick sheet of carbons, rolled in tubes, have been very exciting developments with many applications in medicinal and industrial fields [18]. One of the most important uses of carbon nanotubes is in cancer treatment. Single wall carbon nanotubes can be used as a drug delivery vehicle with high surface area to deliver chemotherapy drugs to the tumor cells and later these purely carbon-made nanotubes can be excreted out of the body by biliary pathway without causing any toxicity [19]. Carbon nanotubes to this point are synthesized from purified petroleum products like methane, benzene, acetylene, and etc. However, camphor can be the environment-friendly, alternative new option [18]. Camphor is a botanical hydrocarbon which is very cheap and can be easily cultivated without fear of shortages unlike petroleum products. Therefore, camphor is an excellent carbon source for the production of a high yield, high purity, and high efficiency carbon nanotubes in the future [20].

The essential oil of Cinnamomum camphora and some other aromatic camphor containing plants, like sage, rosemary and basil which are widely used in traditional medicines, contain monoterpenes. The studies have shown that some essential oils components, especially monoterpenes have suppressive and anti-mutagenic effect in number of human cancer cells including colon cancer, gastric cancer, liver tumor, breast cancer, leukemia and others [21]. Most cancer chemotherapy treatments are highly cytotoxic drugs against proliferating cancer cells as well as healthy cells which can be harmful for the body. With a different mechanism of action, essential oils with their monoterpene components can have multiple pharmacological tumor-suppressive activities, mostly without such harm [22]. Many studies have been done about the various applications and benefits of camphor in pharmaceutical, industrial and environmental fields. Camphor has been used traditionally for many years as a remedy for the relief of pain, inflammation and irritation in body and skin. Recent studies have more focus on the role of camphor in preventing and curing serious and life threatening diseases, when is used purely or combined with other treatments. The study on some species in the Lauraceae family, shows that a number of extracts have significant antioxidant, anti-inflammation and anti-tumor activities [7, 21, 25].These studies with

valuable information indicate that Lauraceae tree species and other camphor containing plants could have very important potential nutraceutical and pharmaceutical applications in the future [25], taking medicine just another step forward.

IV. Dosage and Toxicity

Camphor like any other medication should be used for certain patients with the indicated dosages and contraindications [3].The concentration of 3% to11% has been approved by the FDA for topical use as a pain reliever and anesthetic [23]. Camphor and other terpenoid compounds do not accumulate in the environment since many soil bacteria like Pseudomonas putida readily degrade these compounds [24]. Although herbal medicines and essential oils have been widely used in folk and modern alternative medicine for many years and have shown to be very effective in curing many symptoms and diseases, the misuse of them can be very harmful for the body causing serious problems [22]. Camphor intoxication has been reported in humans and especially children but mostly because of accidental ingestion or exceeding the recommended amount [3].

V. Conclusion

The objective of this paper has been the recent advancement in the exploration of Cinnamomum camphora as phytotherapy and to illustrate its potential as a therapeutic agent. Camphor which has been used traditionally for many years, solely or in combination with other treatments for the relief of pain, inflammation and irritation in body and skin also can be very effective in treating and preventing some serious, life threatening diseases. Considering the growing number of cancer patients, Cinnamomum camphora and its components should be investigated further as a viable option in the treatment of different types of cancer. In addition, more studies on the application of camphor for patients with memory disorders and brain dysfunctions such as in Alzheimer's and Autism are needed. It must be kept in mind that clinicians should remain cautious until more definite studies demonstrate the safety, quality and efficacy of Cinnamomum camphora. For these reasons, extensive pharmacological and chemical experiments, together with human metabolism should be focus of our next studies and further potential of Cinnamomum camphora to be employed in new therapeutic drugs and provide a basis for future research on the application of medicinal plants.

VI. Abbreviations

NOD: Non-obese diabetic; CLE: Camphora leaves extracts; AT: Atrazine; RIPs: Ribosome inactivating problems; CNT: Carbon nanotubes.

Competing Interest

The authors declare that they have no competing interest.

Author's Contributions

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Group1: Conception and design, Analysis and interpretation of data
Group 2: Critical revision of the article
Group 3: Final approval of the version to be published

Soheila Hamidpour
Group1: Analysis and interpretation of data
Group 2: Critical revision of the article
Group 3: Final approval of the version to be published

Mohsen Hamidpour
Group1: Analysis and interpretation of data
Group 2: Critical revision of the article
Group 3: Final approval of the version to be published

Mina Shahlari
Group1: Acquisition of data
Group 2: Drafting the article
Group 3: Final approval of the version to be published

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Figure # 1. Camphora in Public Botanical Gardens, South Australia
Figure # 2. Camphor Laurel, Australia
Figure # 3. Camphor Chemical structure

Figure 1 : Camphora in public Botanic Gardens in Adelaide, South Australia

Figure 2 : Camphor Laurel in fruit at Turramurra railway station, Australia

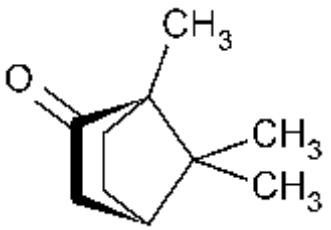

Figure 3 : Camphor, chemical structure, molecular formula

Formula: C10H16O
Melting point: 347°F (175°C)
Density: 990.00 kg/m^3
IUPAC ID: 1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one
Molar mass: 152.23 g/mol

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Effect of Diallyl Disulphide on Protein and Lipid Glycation, and Lipid Peroxidation in Brain of Alloxan Diabetic Rats

By Vijay V, Vickram & Kashinath R T
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Abstract- Non enzymatic glycosylation of proteins and lipids is the main initiating factor for the pathophysiology of chronic diabetic complications. This glycation is more prevalent in insulin independent tissues like brain, kidney, RBCs, etc. Diallyl disulphide (DADS), the principle compound of garlic oil, is well known for its antihyperglycemic, antihyperlipidemic, anticarcinogenic and antibiotic properties. Hence a study was undertaken to assess the anti-glycation properties of DADS, in alloxan diabetic brain tissue, thereby to establish any usefulness of DADS in prevention of central nervous system complications in diabetes mellitus like diabetic dementia or diabetic encephalopathy. The current study showed a significant decrease ($p<0.001$) in glycated proteins, glycated lipids and total TBARS levels in brain tissue of DADS treated diabetic rats as compared to diabetic control rats. Hence it can be concluded that DADS helps in reducing glycation of brain proteins and lipids as well as lipid peroxidation and thus may be useful in prevention of CNS diabetic complications like diabetic encephalopathy.

Keywords: *diallyl disulphide, protein glycation, lipid glycation, diabetic encephalopathy.*

GJMR-B Classification : *NLMC Code: WD 200, WL 348*

Strictly as per the compliance and regulations of:

Effect of Diallyl Disulphide on Protein and Lipid Glycation, and Lipid Peroxidation in Brain of Alloxan Diabetic Rats

Vijay V [α], Vickram [σ] & Kashinath RT [ρ]

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Keywords: diallyl disulphide, protein glycation, lipid glycation, diabetic encephalopathy.

I. Introduction

Non enzymatic glycosylation of proteins and lipids will be normally proportional to available free glucose in the tissues. It can be expected that a consistent hyperglycemia in diabetic subjects may induce hyperglycation of tissue proteins and lipids, and this is high in tissues which are not dependent on insulin for glucose transport like kidney, brain, RBCs, optic lens, etc [1-3]. It is shown that the main initiating factor for the pathophysiology of chronic diabetic complications like diabetic nephropathy is non-enzymatic glycosylation of kidney proteins and lipids[4]. There are evidences for glycation to occurs in brain tissue of diabetic animals like studies of Miyazawa A[5] have established increased lipid glycation in neurons of diabetic animals whereas studies of Jingsheng H[6] have similarly established protein glycation in brain of diabetic rats. Few studies have shown that lipid glycation occurs faster than protein glycation[7]. Since brain has rich content of lipids, lipid glycation is of significance in diabetes induced CNS complications. Glycation of proteins and lipids probably results in increased formation of advanced glycation end products (AGEPs) and advanced glycated lipid products (AGLPs), which leads to formation of various oxidants (like lipid peroxidation products, example Malonaldehyde, etc) resulting in tissue damage[8-12]. These AGEPs and AGLPs are indicated in late diabetic CNS complications like Alzheimers disease[13], diabetic dementia[5] and diabetic encephalopathy[14].

Among the various biological activities of the medicinal plants, the hypoglycaemic and hypolipidemic activities have been the most commonly studied. Garlic, (Allium sativum Linn) is well known for its antidiabetic, antihyperlipidemic, antiatherogenic as well as anticarcinogenic properties[15-18]. DADS, the principle sulphur compound of garlic is probably responsible for the above mentioned beneficial functions of garlic. Studies have shown that DADS crosses blood brain barrier[19,20] and its use in various neurological disorders have been established[21,22].

Hence a study was undertaken to assess the anti-glycation properties of DADS on brain proteins and lipids in alloxan diabetic rats, thereby to establish the usefulness of DADS in prevention of CNS complications in diabetes mellitus like diabetic encephalopathy.

II. Materials and Methods

Alloxan and Diallyl disulphide (DADS) were procured from Sigma Chemical Company. All other chemicals employed were of analytical grade.

Albino rats of both sexes, weighing 300-350g were randomly selected from Central Animal House, BMCH, Chitradurga and were used for the present investigation. The animals were maintained on a standard rat feed supplied from Amrut rat feeds, Bangalore. The experiments were conducted according to the norms approved by Ministry of Social Justice and empowerment, Government of India, and Institutional Animal Ethics Committee (IAEC) guidelines. The animals were fasted overnight and Diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan (150mg/kg body wt.)[23], in sterile normal saline. The animals were considered diabetic if their blood

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glucose were consistently above 300mg/dl and urine showed consistent glucosuria. The treatment was started on 5th day after alloxan injection and was considered as first day of treatment. The rats were divided into three groups comprising six rats in each group as follows:

Group I: Normal rats – which were fed on 30 ml of normal saline per kg body weight, through gastric intubation, daily for 90 days.

Group II: Diabetic Control rats - which were fed on normal saline 30ml / kg body weight, through gastric intubation, daily for 90 days.

Group III: Diallyl disulphide (DADS) treated Diabetic rats – which were fed on DADS (100mg/ kg body weight) prepared in normal saline, given as 30ml / kg body weight suspension, through gastric intubation, daily for 90 days.

On completion of the stipulated period, the rats were anaesthetized by anaesthetic ether and were sacrificed by cervical dislocation. Blood was collected in heparinized tubes from internal jugular vein. Whole brain was dissected and net weight was noted. Immediately the brain was processed as follows. One part of whole brain was homogenized with 9 parts of cold Phosphate buffer (pH 7.4) using Potter Elvehjam homogeniser and the extract was used for estimation of total proteins[24] and carbohydrate content of these protein [Glycated protein][25]. A second part of brain was homogenized with 9 parts of Chloroform methanol (1:1 v/v) mixture using Potter Elvehjam homogeniser and the extract was used for total lipids[26] and carbohydrate content of this lipids [Glycated lipids][25]. And another part of whole brain was homogenized with 9 parts of trichloroacetic acid (10%) and extract was used for the estimation of thiobarbituric acid reactive substances (TBARS) levels[27]. Whole blood was employed for glycated hemoglobin estimation[25]. A part of whole blood was centrifuged at 3500 rpm for 6-8mins and the free separated plasma was used for glucose estimation[28]. The free sugar content of phosphate buffer extract was estimated by Folin Wu method[29] and the value obtained was deducted from the total carbohydrate content of phosphate buffer protein to calculate glycated protein content.

The results were expressed as mean $\pm$ SD. Statistical analysis was done by using student "t" test.

III. Results

The results obtained are given in table 1 and 2. Table 1 gives the glycated Hb levels, plasma glucose levels, body weight and ratio of brain to body weight in normal rats (group 1), alloxan diabetic rats (group II), as well as in DADS treated alloxan diabetic rats (group III).

As seen from the table, there is a significant increase in plasma glucose levels ($p<0.001$), glycated hemoglobin levels ($p<0.001$) body weight and ratio of brain to body weight ($p<0.001$) in group II as compared to group I rats. A significant decrease is seen in the above parameters ($p<0.001$) in group III rats as compared to group II rats. Further no significant alteration is observed in plasma glucose levels in group III rats as compared to group II rats.

Tables 2 shows the levels of brain tissue total proteins, glycated brain proteins, brain tissue total lipids and glycated brain lipids in group I, group II and group III rats. A significant raise in glycated brain proteins ($p<0.001$), glycated brain lipids ($p<0.001$) and brain total lipids ($p<0.001$) were observed in group II rats as compared to group I rats whereas a significant decrease in brain total proteins ($p<0.05$) was observed in group II as compared to group I. A significant decrease in glycated brain proteins ($p<0.001$), glycated brain lipids ($p<0.001$) and brain total lipids ($p<0.001$) is observed in DADS treated diabetic rats (group III) as compared to diabetic control rats (group II).

IV. Discussion

In the present study, administration of alloxan (150mg/kg body weight) induced hyperglycemia in the albino rats as evidenced by elevated plasma glucose levels and glycated hemoglobin levels in group II rats (refer table 1). The levels of glycated hemoglobin have been shown to be an important parameter of chronic glycemic control in diabetes. The decrease in body weight of diabetic rats is due to increase in the protein catabolism mainly in skeletal muscles that helps to channel amino acids for gluconeogenesis, decrease in protein uptake as well as insulin deficiency induced lipolysis[30].

There is substantial epidemiological evidence that, besides the long-term complications of diabetes mellitus, which include accelerated atherosclerosis, retinal microvascular damage, renal failure caused by glomerular injury, and peripheral neuropathy, the disease also has multiple effects on the central nervous system. Diabetic patients have at least twice the risk of stroke[31] and may show performance deficits in a wide range of cognitive domains[32]. The mechanisms underlying this gradually developing end-organ damage, known as diabetic encephalopathy, are only partially understood and can involve vascular changes and direct damage to neuronal cells by glucose[33, 34]. Although the high level of glucose in the brain cortex of diabetic rats has been questioned[35], it has recently been reported that glucose levels increase by up to three times in the hippocampus of diabetic rats compared with controls[36]. Emerging evidence suggests that increased glycation leads to the overproduction of superoxide by the respiratory chain and consequent oxidative stress play a role in the pathogenesis of diabetes complications[14].

Many studies have shown that garlic and its compounds exhibit diverse biological activities like anti-

tumorigenic, anti-atherosclerosis, detoxification, anti-inflammatory, antioxidant etc.[21,37,38]. Also, garlic oil-derived organosulfur compounds such as diallyl trisulphide, diallyl disulphide, and diallyl sulphide provide significant protection against carcinogenesis, and this protection is likely related with their antioxidant properties[39]. Moreover, the lipophilic characteristics of these compounds allow crossing the blood-brain barrier as follows: dialyl sulfide crosses the blood-brain barrier easier than diallyl disulphide > diallyl trisulfide > S allylcysteine[20,22].

DADS, the principle sulphur compound of garlic oil is well known to possess hypoglycemic, hypolipidemic action[15,16] as well as anti-glycation activity[4,40] . It is known that DADS may enhance the half life of insulin probably by decreasing the activity of insulinase enzyme by a sulphydryl exchange reaction[41]. The results given in table 2 indicates, the glycated protein and lipid levels in brain are significantly decreased in DADS treated diabetic rats as compared to diabetic control rats suggesting that DADS may interfere in the non-enzymatic glycation process. This in part may be due to increased glucose oxidation or due to decreased gluconeogenesis, hence resulting in lesser availability of glucose, thus lowering glycation, as DADS has been suggested to possess hypoglycaemic action.
DADS is a disulphide, may be involved in sulphydryl exchange reactions with proteins or enzymes[42,43] similar to any other disulphide as follows:

R1- S-S-R1 + R2 –SH -------- > R1-S-S-SR2 + R1- SH

Such non-enzymatic glycation in tissue proteins and probably in tissue lipids may induce an alteration in three dimensional structure of tissue proteins and thereby making the protein thiol (-SH) groups vulnerable for oxidative damage[44]. DADS decreases tissue protein glycation as well as tissue lipid glycation, thereby may decrease sulphydryl protein/lipid oxidation and hence preventing the possible tissue damage. This is evidenced by a decrease in brain tissue TBARS levels in DADS treated diabetic rats as compared to alloxan diabetic control rats (refer table II).

V. Conclusion

The present study suggests that DADS reduces glycation of brain protein and lipids as well as lipid peroxidation in alloxan diabetic brain tissue thus may be effective in prevention of CNS complications in diabetes mellitus like diabetic encephalopathy, diabetic dementia, etc.

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Table 1 : showing the plasma glucose levels, glycated hemoglobin levels as well as body weight and brain to body weight ratio in Group I, Group II and (Goup III) rats

	Plasma glucose (mg/dl)	Glycated Hb (%)	Body weight (Gms)	Brain wt / body weight ratio
Group I (n=6)	112.26 ± 19.6	3.9 ± 1.2	323.81 ± 55.65	0.0062 ± 0.004
Group II (n=6)	623.66*** ± 102.08	16.2*** ± 1.5	217.85**** ± 31.40	0.0087* ± 0.005
Group III (n=6)	565.00 ± 135.01	12.5*** ± 1.9	210.16 ± 50.32	0.0089 ± 0.004

Note: 1. Number in parentheses indicate the number of animals in each group.
2. The values are expressed as their mean ± SD
3. Significance level * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2 : showing brain tissue total proteins, glycated proteins, total lipids, glycated lipids and brain tissue total TBARS levels in Group I, Group II and Group III rats

	Brain Total Proteins (mg/g)	Brain Glycated Protein (%)	Brain Total Lipids (mg/g)	Brain Glycated Lipids (%)	Brain Tissue TBARS (μ mol/g)
Group I (n=6)	85 ± 21.21	8.26 ± 0.98	62.18 ± 4.09	6.49 ± 2.22	7.12 ± 1.67
Group II (n=6)	75 ± 15.43	9.97**** ± 0.98	78.30**** ± 12.66	27.98**** ± 5.06	13.17**** ± 2.13
Group III (n=6)	*75 ± 15.43*	*9.12** ± 1.37*	*77.57 ± 6.54*	*17.66**** ± 1.59*	*9.34**** ± 1.88*

Note: 1. Number in parentheses indicate the number of animals in each group.
2. The values are expressed as their mean ± SD
3. Significance level * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

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Sequential Analysis of Postural Control Resource Allocation During a Dual Task Test

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Abstract- Objective : To investigate postural control factors influencing automatic (reflex-controlled) and attentional (high cortical) factors on dual task.

Method : We used a dual-task model to examine attentional factors affecting control of posture, subjecting test subjects to vibration stimulation, one-leg standing and verbal or nonverbal task trials. Twenty-three young, healthy participants were asked to stand on force plates and their centers of pressure (COP) were measured during dual task trials. We acquired 15 seconds of data for each volunteer during six dual task trials involving varying task combinations.

Results : We observed significantly different sway patterns between early and late phases of the dual task trials that probably reflect attentional demands. Vibration stimulation perturbed sway more during the early than the late phases; with or without vibration stimulation, the addition of secondary tasks decreased sway in all phases, and greater decreases in sway were observed in late phases when subjects were assigned nonverbal tasks. Less sway was observed during nonverbal task in a sequential study.

Keywords: *task performance, analysis, postural balance, attention.*

GJMR-B Classification : *NLMC Code: QV 37, QV 55*

Strictly as per the compliance and regulations of:

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Conclusion : The attentional and automatic factors were analyzed during a sequential study. By controlling the postural control factors, optimal parameters and training methods might be used in clinical applications.

Keywords: task performance, analysis, postural balance, attention.

I. Introduction

Sensory perturbations of visual, somatosensory, and vestibular systems disrupt postural stability. Postural control can be influenced by automatic (reflex-controlled) and attentional (high cortical) factors, and previous studies have suggested that postural control systems require varying degrees of attention depending on the postural tasks involved and the age of the subjects.[1-5] Attentional factors are thought to arise from the central nervous system (CNS), while automatic factors are reflex-controlled by somatosensory (muscle, skin and pressure receptors), visual and vestibular inputs.[6,7]

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Dual task paradigms are important tools for understanding balance control. The primary task is usually postural control, which involves standing on a force plate with different levels of difficulty, for example on an uneven surface or standing on one leg. Teasdale *et al.* [8] showed that adults of all ages exhibit delays in reaction time as postural task complexity increases. When vibrations are applied during primary postural tasks they typically cause directional shifts in subjects, due to increasing primary (Ia) afferents that are discharged during vibration and interpreted as lengthening of the vibrated muscles.[7] In previous studies, tendon vibration stimulation was shown to increase postural sway, and subjects frequently experience vibration-induced compensatory losses of balance, falling in the same direction as the applied vibration.[9,10] However, in several studies directional shifts were either increased or decreased according to stimulation intensity and type.[11-15]

The secondary task in dual task paradigms is usually attention demanding, and task intensity and difficulty influence postural control in various ways.[5,16,17] Both verbal and nonverbal tasks have been applied as secondary tasks in dual task paradigms.[18,19] Verbal tasks are considered relatively easy for participants to complete, while nonverbal tasks are more difficult due to their attention demanding characteristics. Verbal and non-verbal working memory are thought to be associated with different regions of the brain.[20-22]

Several studies have explored the effects of tendon vibration on postural control and the ability to complete tasks. However, the relationships between these parameters and postural sway have not been investigated in a dual task study design. Furthermore, the sequential relationship between automatic control and attention factors during in dual task contexts is still unclear.

We examined the sequential relationships of dual task on postural control. When subjects were subjected to dual task trials, we were able to sequentially observe the demands of attention factors, how they differed depending on the combinations of tasks that were presented, and the effects of attentional factors on balance control. The clinical implications of postural control can be understood through dual task performance and resource allocation analysis.

II. Materials and Methods

Subjects

Twenty-three young, healthy participants participated in this study. No participants reported neurological or orthopedic disorders, and none were receiving medications known to affect postural control. All participants provided informed consent prior to testing.

Methods

A total of 23 subjects participated in the study. Detailed demographic data are shown in Table 1. All participants were randomly assigned to six trials (Table 2). Three participants were excluded due to poor compliance and the other 20 participants were included in experiments.

Each participant stood on his or her dominant leg on a force plate while watching a computer display monitor. While standing on the force plate, each participant was subjected six successive dual task trials in random order (Table 2). Secondary tasks were given to participants via the computer display and center of pressure (CoP) values were recorded during each experiment.

Subjects were asked to stand with arms folded and a button in one hand, and to press the button to indicate correct answers to task questions, in order to reduce any confounding effects of articulation.[23] All participants were allowed two practice trials, and each trial began with the 'ready' cue, followed three seconds later by the 'set' cue. When the participants were told to be 'ready', they stood on one leg and held that position for 15 seconds. Participants rested for one minute between trials.

A primary task involving proprioceptive vibration stimulation was given to subjects as they stood on one leg for 15 seconds. A vibratory motor (consisting of two vibratory plates: 10 gram, 10 mm) was applied to the skin overlying the Achilles tendon and tibialis anterior on the inferior third of the dominant leg. The vibratory motor (Jahwa Co., Seoul, Korea) produced a stimulus at 8000 rpm and 10 mm of motor diameter. The amplitude and intensity of vibration were controlled by NI LabVIEW 8.0 software. As soon as participants could feel the vibratory stimulation, we determined and set it at supra-threshold intensity.

Leg dominance was determined by ball kick tests. During the one-leg stand, the CoP was measured and recorded by a Bertec force plate® (Bertec Inc., Columbus, USA) system that consists of four road cells and Acquire software version 5.1.

A cognitive task was presented to subjects either as a series of random characters (verbal) or shapes (nonverbal) displayed on a computer screen. If the presented character or shape was identical to the one that had been displayed two steps before (two back task), participants were asked to respond by pressing the button.[24,25] Cognitive performance was calculated by the number of correct responses and the response time. Sway was measured by the force plates and amplified, and the summation of the sway distance during each trial was recorded as the distance from the center of pressure (DCP). Three trials of DCP data were acquired per each test (trial I~VI), and the mean values were calculated for use in analyses. The recorded force information was used to derive the position time function of the CoP for each trial.

Statistical analysis

Data were collected during three sessions performed on three different days no more than a week apart. Acquire software was used to receive signals from the force plate, and Matlab software (The MathWorks Inc., Natick, USA) was used to analyze the data after filtering with the Butterworth method.[26-27]

Experimental parameters included vibration (on, off) and task context (no task, verbal task, nonverbal task). The total summations of sway distances of 15 seconds from six different trials each were compared using paired t-tests. Participant performance under different test conditions (no secondary task, verbal/nonverbal tasks without vibration and without secondary task, and nonverbal/verbal tasks with vibration) were compared using one-way ANOVA with a Bonferroni correction. The amount of sway in time sequence was analyzed using one–way repeated ANOVA. After first one second for one leg standing adjustment, the summation of sway distance in the first phase (2~8 seconds) and late phase (9~15) were compared using paired t test. To analyze the sway difference in time sequence, the sway differences in each second were acquired and compared with the first one second sway difference (2 ~3 second) using a paird *t* test.

III. Results

The total summation of the DCPs is shown in Fig. 1. Participants exhibited more sway when subjected to vibration (434.99±86.73 mm) than in trials with no vibration (416.54±70.97 mm). The addition of verbal and nonverbal secondary tasks decreased sway compared to trials in which participants were not given secondary tasks. We did not observe any significant differences between trials when secondary tasks were provided with or without vibration stimulation.

However, during trials in which participants are asked to maintain one-leg stands, greater postural control is needed as time goes by to maintain balance and eventually the participants were forced to break posture. In our study, we observed that after 8~10 seconds, sway began to increase in such trials. In addition, when secondary tasks were given to standing participants, noticeable changes of sway were observed after 8~10

seconds (Fig. 2). Therefore, we split the trials into early and late phases with the distinction made when we observed noticeable sway differences (Fig. 3, 6).

a) Vibration stimulation study

One-leg standing tasks and/or vibration stimulations were given to participants as primary tasks. More sway was noted when supra-threshold intensity vibration stimulations were applied than in trials with one-leg standing alone (Fig. 2). Significant increases in sway were noted during early phases, but none were observed in the late phases. After an adjustment period with one leg standing (1~2 seconds), more sway was noted during vibration trials than in trials requiring participants to maintain one-leg standing alone, up to 9 seconds However, after 9 seconds, we did not observe any significant differences in sway between trials. In one-leg stand trials, after a short adjustment period with one leg standing, more sway was noted during late phases compared to early phases. In trials combining vibration and one-leg standing, more sway was noted in early phases but then decreased in late phases. The greatest amounts of sway were noted at the end of both types of trials (14 ~ 15 seconds).

b) Secondary tasks given to subjects standing on one leg

Sway decreased when subjects standing on one leg were given each secondary task to complete (Fig. 3). We observed significant decreases in sway during the late phases of verbal task trials when compared to trials in which participants were not given secondary tasks. Compared to trials in which participants were given verbal tasks, decreased sway was noted for all phases during nonverbal task trials, but this difference was not significant. Significant decreases in sway were noted for all phases of nonverbal task trials compared to trials in which participants were not given secondary tasks.

c) Vibration stimulation applied to subjects given secondary tasks

For trials in which subjects were given nonverbal tasks, the application of vibration stimulation increased sway in the late phases, though this increase was not significant (Fig. 4). In trials in which participants were given verbal tasks, the application of vibration stimulation increased sway in the late phases, but this increase also was not significant. In a general sense, vibration did not increase sway when secondary tasks were given in any phases (Fig. 5).

d) Secondary tasks given to subjects standing on one leg standing and exposed to vibration stimulation

The assignment of secondary tasks decreased sway in all phases in subjects standing on one leg and simultaneously exposed to vibration stimulation (Fig. 6). Trials in which subjects were given both verbal and nonverbal tasks resulted in significantly decreased sway in all phases compared to trials with no secondary tasks. Compared to trials in which participants were given verbal tasks, nonverbal task trials resulted in significantly decreased sway within an early phase.

IV. Discussion

The present study implemented a difficult, attention-demanding two-step recall memory task. Postural changes were less apparent in subjects given attention-demanding tasks than in subjects given a primary task only. The effects of attention-demanding tasks are similar to those of external foci. Attention-demanding tasks divert attention away from postural control, perhaps allowing for more automatic processes and less conscious interference in the control of balance.[28] Previous studies have suggested that requesting participants to focus on body sway induced an increase in sway and hampered neuromuscular efficiency for controlling posture during standing.[29,30] This phenomenon has been explained by either high cortical arousal[5,16] or automatic reflex caused by the total consumption of attention factors.[4,5]

Previous studies suggested that stimuli used to test verbal and nonverbal working memory are received and interpreted by different regions of the brain. Based on neural networks, it has been suggested that the verbal/non-verbal dichotomy reflects ventral/dorsal or left/right domain differences in the brain.[20-22] Prominent activation of the left hemisphere is associated with verbal coding while right prefrontal activation is associated with nonverbal coding.[20] Therefore, differences in the area of cortical stimulation targeted by different tasks may also be related to body sway. In our study, in non-verbal task trials, which are presumably related to right prefrontal activation, directional shifts were less apparent. Perhaps right prefrontal activation allows for more automatic processes to activate and control balance without conscious interference. On the other hand, in verbal task trials, which are presumably related to left hemisphere activation, fewer automatic processes may take place.

We found that the application of vibration stimulation induced sway, especially in the early phases during which more automatic factors are activated. This may reflect an increase in body awareness due to the application of supra-threshold degrees of vibration stimulation. McIlroy *et al.*[17] hypothesized that the processing requirements for postural control vary during the time course of stability recovery and that therefore the related attentional demands also vary. The characteristics of the time courses predicted by stability recovery theory are very intriguing, and it is not clear whether the results of McIlroy's study can be generalized to the control of human posture.[5] Furthermore, no studies have evaluated each phase in sequence after several seconds of postural control.

We observed postural control response during a total period of 15 seconds per trial. We hypothesized that the action of attentional factors becomes more important as time goes on and that body awareness increases after an initial adjustment phase. This is similar to the summation of McIlroy's three phases. We divided each trial into early and late phases. After 8 seconds, the amount of sway in time sequence was significantly increased. From 0 to 8 ~10 seconds, the early phase includes the characteristic adjustment time for one leg standing and our results implicate the involvement of more automatic reflexes during that phase, as stance does not seem to be disturbed by attentional factors. The late phase starts after 10 seconds, during which attention factors become more prominent. When participants were given difficult memory tasks to complete, sway greatly decreased compared to the performance of participants who were not given such tasks (Fig. 2, 3, 6). From these results, it seems possible that postural response can be divided into an early phase and a more attention-demanding late phase.

We attempted to induce changes in postural control resource allocation by implementing dual task paradigm comprised of a postural task (one leg standing, vibration stimulation with supra-threshold intensity) and a high demand cognitive working memory task (two-word recall). Cognitive resources play a key role in maintaining postural stability in older adults, which may be due to an age-related decline in sensory and motor function.[8,31-33] In previous studies, older adults are characterized as giving greater priority to the task that they perceive to have greater importance.[34] Given a choice between postural control and a cognitive task, older adults prioritized the former[35] due to the high prevalence of instabilitiy and risk of falling in the elderly.[34] In one study, young adults did not show a decrease in postural sway for either easy or difficult balance tasks.[36] However, Swan *et al.*[36] demonstrated a decrease in postural sway in older adults during difficult dual task balance conditions, but no sway reduction for relatively easy balance tasks. Since demanding tasks impose greater cognitive loads for older adults than younger adults, older adults may be better subjects in which to evaluate changes in postural control resource allocation.

We observed the sequential influence of automatic and attention factors in dual task paradigms in young participants. Our results suggest that optimal training strategies for patients at high risk of injury from falls, such as older adults, should prioritize automatic factors and the maintenance of external focus over postural control.

The shortcomings of our study include a relatively small sample size and broad vibration stimulation levels that were not sensitive enough to assess the differing effects of varying, sub-threshold vibration stimulation intensities. Future studies that include more subjects and more standardized levels of difficulty may demonstrate clearer results. Future research should focus not only on a better understanding of dual task on postural control in time sequence, but also on their detailed applications in various rehabilitation settings.

In conclusion, both the automatic and attentional factors are required for postural control. We observed that the attentional factors were prioritized for postural control and more dominant in the later phase during a sequential study. By controlling the postural control factors, optimal parameters and training methods for postural control can be designed for use in practical applications.

V. Conflict of Interest

No potential conflict of interest relevant to this article was reported.

VI. Acknowledgments

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Table 1 : The Demographic Data of the Participants

	Verbal task	Nonverbal task
Sex(male/female)	11/9	
Age(year)	28.36±4.03	
Height(cm)	169.05±7.26	
Weight(kg)	59.94±12.16	
Accuracy of correction (%)		
Vibration off	0.84±0.09	0.78±0.14
Vibration on	0.83±0.09	0.80±0.12
Reaction time(ms)		
Vibration off	531.24±74.43	508.50±77.35
Vibration on	512.53±94.54	516.31±82.72

(p<.05)

Table 2 : Six Trials of Dual Task

Name	Abbreviations	Condition	Sequence
Trial I	S	One leg standing	Randomized
Trial II	Sv	One leg standing+verbal task	Randomized
Trial III	Snv	One leg standing+nonverbal task	Randomized
Trial I-1	viS	One leg standing+vibration on foot	Randomized
Trial II-2	viSv	One leg standing+vibration on foot+verbal task	Randomized
Trial III-3	viSnv	One leg standing+vibration on foot+nonverbal task	Randomized

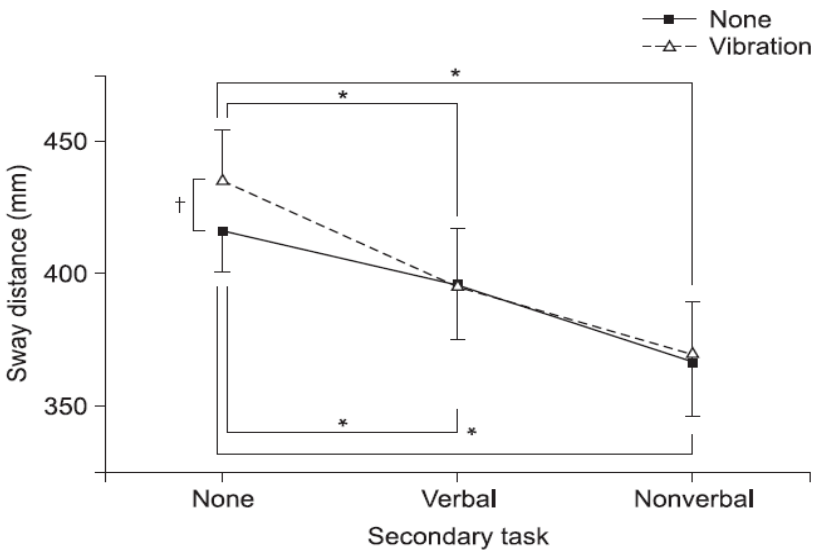

Figure 1 : Comparison among the total summation of distance of center of pressure during 6 trials.

- *Denotes significant differences between different secondary tasks (none and verbal, none and nonverbal).
- †Denotes significant differences between none and Vibration.

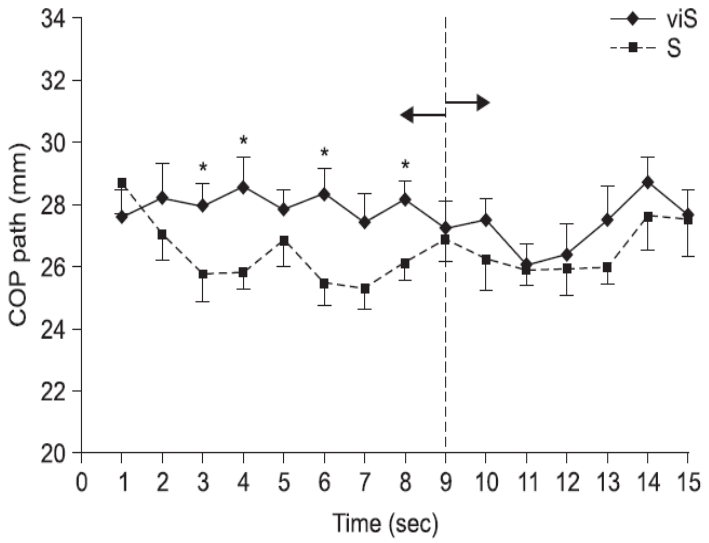

Figure 2 : The amount of sway per second with or without vibration during one-leg standing trials.

- viS stands for vibration and one-leg standing as the dual task.
- S stands for one-leg standing only as the primary task.
- COP stands for the center of pressure distance.
- Denotes significant differences between viS and S.

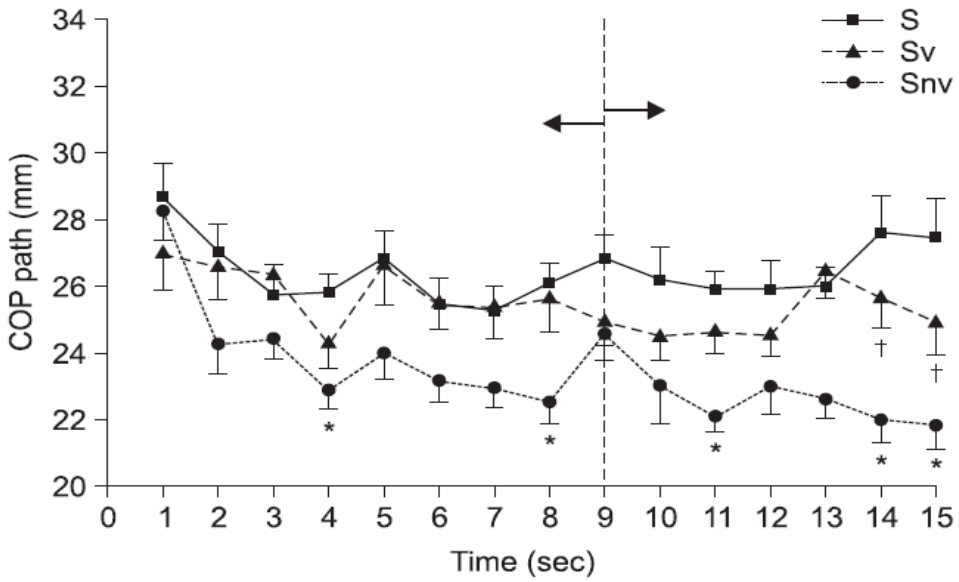

Figure 3 : The amount of sway per second with secondary tasks during one-leg standing trials.

- S stands for one-leg standing as the primary task.
- Sv stands for one-leg standing as the primary task and a verbal task as the secondary task.
- Snv stands for one-leg standing as the primary task and a nonverbal task as the secondary task.
- Denotes significant differences between Sv and Snv.
- †Denotes significant differences between viS and viSv.

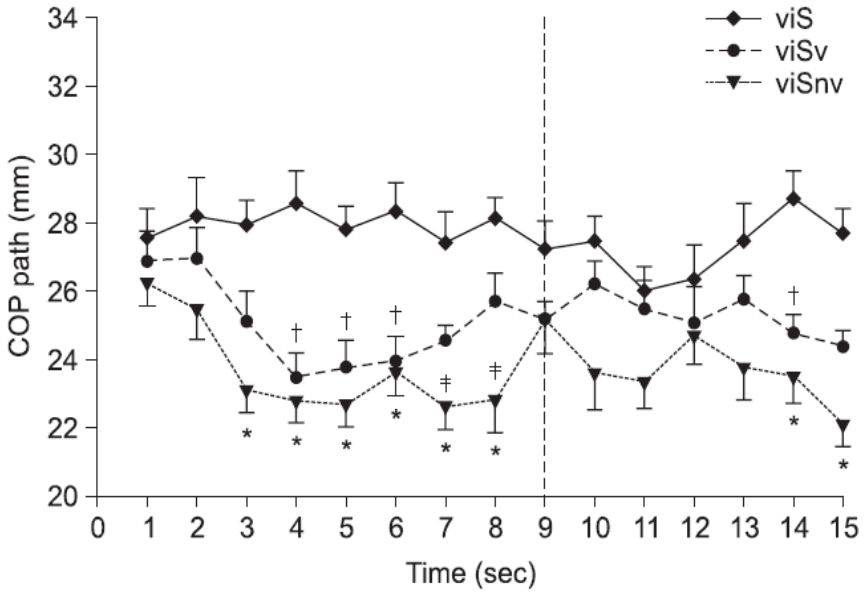

Figure 4 : The amount of sway per second with secondary tasks during one-leg standing trials with vibration.

- viS, stands for vibration and one-leg standing as the primary task.
- viSv, stands for vibration and one-leg standing as the primary task and a verbal task as the secondary task.
- viSnv, stands for vibration and one-leg standing as the primary task and a nonverbal task as the secondary task.
- *Denotes significant differences between viS and viSnv.
- †Denotes significant differences between viS and viSv.
- ‡Denotes significant differences between viSv and viSnv.

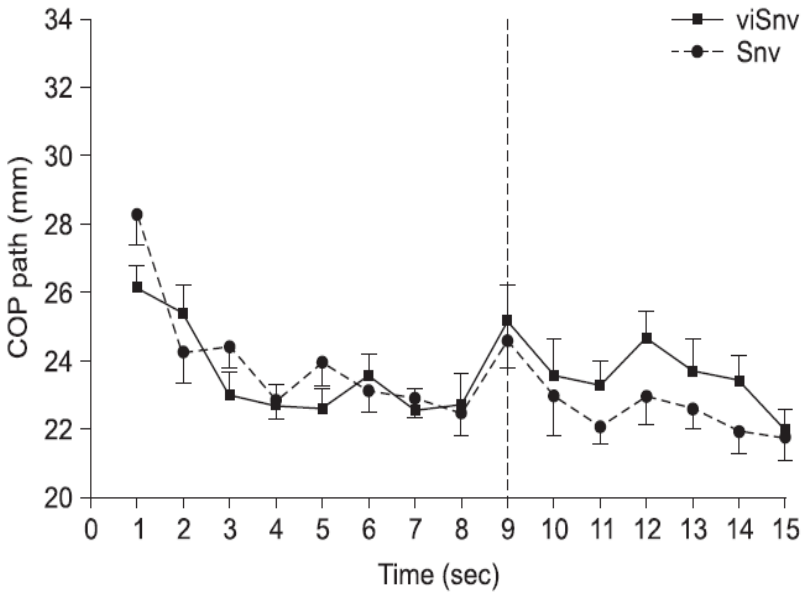

Figure 5 : The amount of sway per second with or without vibration during one-leg standing trials with nonverbal task.

- viSnv, stands for vibration and one-leg standing as the primary task and a nonverbal task as the secondary task.
- Snv, stands for one-leg standing as the primary task and a nonverbal task as the secondary task.

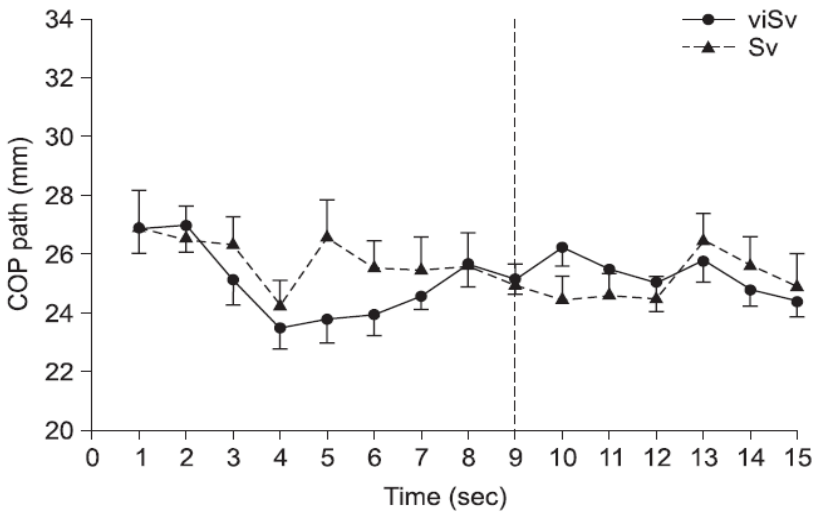

Figure 6 : The amount of sway per second with or without vibration during one-leg standing trials with verbal task.

- viSv, stands for vibration and one-leg standing as the primary task and a verbal task as the secondary task.
- Sv, stands for one-leg standing as the primary task and a verbal task as the secondary task.

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Drug-Like Properties of some Esters of *Ortho-/Meta-/Para*-Alkoxyphenylcarbamic Acid Containing *N*-Phenylpiperazine Fragment

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Abstract- In recent years, *in silico* pharmaceutical tools have a notable impact of drug discovery as complementary methods for *in vitro* and *in vivo* assays. Such procedures help to optimize pharmacokinetic and pharmaceutical properties of (not only) drug-like candidates. Following the Lipinski´s Rule of Five concept and experimental partition coefficients data as well, the majority of currently in silico investigated compounds, 8aB–8iB, which structure contained so-called privileged structure, 4-(3-trifluoromethylphenyl)piperazin-1-yl fragment, would be regarded as the drugs with the physicochemical properties that could be convenient in terms of their pharmacokinetic and metabolic profiles. In addition, their ability to cross blood–brain barrier was *in silico* inspected. In general, the CNS drugs tend to be more lipophilic, be less polar, have shown less flexibility, had lower molecular weight and smaller molecular volume as well than the drugs applied for other therapeutic indications. Following the calculated (molecular weight, topological polar surface area, hydrogen-bond acceptors count, hydrogen-bond donors count, rotatable bonds count, CLOGP data) and experimentally estimated (log P_{exp}) readouts, it was suggested that concerned derivatives would probably not cross blood–brain barrier by passive diffusion, thus they could not affect CNS processes.

Keywords: *rule of five, n-arylpiperazines, blood–brain barrier.*

GJMR-B Classification : *NLMC Code: QV 37.5, QU 98*

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Ivan Malík

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I. Introduction

More than ten years have passed since the research paper concerning the Rule of Five (RO5) by Lipinski et al. (2001) was published[1]. Assuming the evaluation of potentially orally active compounds, in the discovery setting, the RO5 predicted[1,2] that poor absorption or permeation was more likely when there were more than five hydrogen-bond donors (OH plus NH count), more than ten hydrogen -bond acceptors (O plus N atoms), the molecular weight (MW) was greater than 500 and the calculated log *P* value for the system octan-1-ol/water using CLOGP approach[3] was more than 5 or higher than 4.15 when applied Moriguchi MLOGP predictive method[4], respectively. In other words, these physicochemical parameters were connected with acceptable aqueous solubility and intestinal permeability and comprised the first steps of oral bioavailability. The RO5 was deliberately created to be a conservative predictor in an area where medicinal chemistry produced too many compounds with poor physicochemical properties[2]. Veber et al.[5] later suggested that the commonly applied MW cutoff at 500 did not itself significantly separate compounds with poor oral bioavailability from those with acceptable values. Taking into account the fact that molecular rigidity was a much more complex issue than the simple counting of rotatable bonds, their conclusions[5] also pointed out that the compounds which met only two criteria, i.e. ten or fewer rotatable bonds and the value of polar surface area (PSA) equal to or less than 140 Å^2 (or 12 or fewer hydrogen-bond donors and acceptors), would have a high probability of good oral bioavailability. Nevertheless, it is important to emphasize the limitations of these rules: (i) the RO5 applies only to the compounds which are delivered by the oral route, (ii) the RO5 applies only to the compounds which are absorbed by passive mechanisms, (iii) there are important exceptions (MW>500 and reduced molecular flexibility and constrained PSA; natural products), (iv) passing the RO5 is no guarantee that a compound is drug-like, (v) the RO5 says nothing about specific chemistry structural features found in drugs or non-drugs[2,6,7].

From chemical viewpoint, currently *in silico* investigated molecules, labelled as **8aB–8iB**, could be regarded as *ortho*-/*meta*-/*para*-alkoxyphenylcarbamic acid-based derivatives as well as *N*-arylpiperazine -based structures (Figure). It was previously found out that some of them (all the investigated molecules were prepared and tested as the salts with hydrochloric acid) have shown relatively promising antimicrobial profile[8,9]. In general, the *N*-arylpiperazine moiety was regarded as so-called privileged structure[10] – it represented a class of the molecules capable of binding to multiple receptors with high affinity, inter alia by displaying key physicochemical characteristics that facilitated their ability to bind to them. The use of such fragment should allow the medicinal chemists to rapidly discover biologically active compounds across a broad range of therapeutic areas[11]. Following mentioned, the principal objective of current paper was to evaluate if these derivatives **8aB–8iB** could potentially exhibit convenient physicochemical properties, which would be

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related to their favorable pharmacokinetic profiles, in terms of RO5.

II. Materials and Methods

Currently *in silico* studied compounds **8aB–8iB**, chemically 3-[4-(3-trifluoromethylphenyl)piperazin-1-yl]--2-hydroxypropyl (2-/3-/4-alkoxyphenyl) carbamates (Figure), were previously *in vitro* antimicrobially screened against Gram-positive *Staphylococcus aureus* ATCC 6538, Gram-negative *Escherichia coli* CNCTC 377/79 and *Candida albicans* CCM 8186, a yeast, respectively, as the salts with hydrochloric acid[8,9], i.e. **8a–8i**, chemically 1-[3-(2-/3-/4-alkoxyphenylcarba-moyloxy)-2-hydroxypropyl]-4-(3-trifluoromethylphenyl)pi- perazinium chlorides. Although they contained stereogenic centre, they were prepared and tested as racemates. The letter „**B**" in the entry (Table) meant the „base".

The data, which characterized molecular weight (MW), hydrogen-bond acceptors count (*n*ON), hydrogen-bond donors count (*n*OHNH), topological polar surface area[12] (TPSA), rotatable bonds count (*n*rotb) and the predicted logarithms of partition coefficient for the system octan-1-ol/water by applying miLogP 2.2 substructure approach, of investigated compounds **8aB–8iB** were calculated by using interactive applet of Molinspiration Cheminformatics software tool (Molinspiration Cheminformatics, Slovak Republic). The CLOGP 4.0 readouts were generated by using ChemBioDraw Ultra 11.0 program package (CambridgeSoft, USA). The outputs of log *P* calculated by Moriguchi method (MLOGP) were obtained by an interactive applet of Virtual Computational Chemistry Laboratory (VCChL)[13]. The ALOGP procedure of log *P* prediction was calculated using VCChL applet based on the approach developed by Ghose and Crippen[14].

The readouts of partition coefficient logarithms, which were experimentally estimated in octan-1-ol/buffer (p*H*=7.0) medium, were adopted from the research paper[15] of Sedlárová et al. (2007).

The observed differences between calculated (CLOGP 4.0, MLOGP, miLogP 2.2 and ALOGP, respectively) and experimentally estimated (log P_{exp}) log *P* values were expressed by the Absolute Average Residual Sums (AARS). If the AARS output was in the range of 0.000–0.490, the MLOGP, CLOGP 4.0, miLogP 2.2 and ALOGP approach, respectively, was qualified as acceptable, while AARS value within the area of 0.500–0.999 indicated that respect predictor tool was viewed as disputable and, finally, if calculated AARS readout exceeded 1.000, it was then classified as an unacceptable predictive procedure[16].

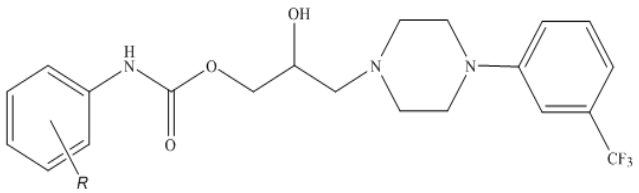

Figure : General chemical structure of currently *in silico* investigated compounds **8aB–8iB**; **8aB**: *R*=2-OCH_3, **8bB**: *R*=2-OC_2H_5, **8cB**: *R*=3-OCH_3, **8dB**: *R*=3-OC_2H_5, **8eB**: *R*=3-OC_3H_7, **8fB**: *R*=4-OCH_3, **8gB**: *R*=4-OC_2H_5, **8hB**: *R*=4-OC_3H_7, **8iB**: *R*=4-OC_4H_9.

Table : The descriptors which characterized currently *in silico* inspected derivatives **8aB–8iB**

Entry	MW	*n*ON	*n*OHNH	*n*rotb	CLOGP 4.0	MLOGP	ALOGP	miLogP 2.2	logP_{exp}
8aB	453.46	7	2	9	4.21	3.16	3.78	3.72	3.57
8bB	467.49	7	2	10	4.74	3.65	4.13	4.10	3.63
8cB	453.46	7	2	9	4.21	3.16	3.78	3.75	3.53
8bD	467.49	7	2	10	4.74	3.65	4.13	4.12	3.61
8eB	481.52	7	2	11	5.27	4.14	4.65	4.63	4.06
8fB	453.46	7	2	9	4.21	3.16	3.78	3.77	3.60
8gB	467.49	7	2	10	4.74	3.65	4.13	4.15	3.71
8hB	481.52	7	2	11	5.27	4.14	4.65	4.65	3.90
8iB	495.54	7	2	12	5.80	4.64	5.11	5.21	3.98

III. Results and Discussion

Presently *in silico* calculated outputs revealed that almost all the inspected molecules completely met the RO5 (Table). In detail, the substances **8aB–8iB** have shown MW less than 500, *n*ON=7 and *n*OHNH=2 as well. The calculation of PSA in a classical way, however, was rather time consuming, because of the necessity to

generate a reasonable 3D molecular geometry and to determine the surface itself. Additionally, such calculations required specialized software to generate the 3D molecular structures and to determine the surface. The methodology for the calculation of used TPSA was described in details in the paper[12]. Briefly, such procedure was based on the summation of tabulated surface contributions of polar fragments (atoms regarding also their environment). These fragment contributions were determined by least squares fitting to the single conformer 3D PSA for 34,810 drugs from the World Drug Index. Topological polar surface area (TPSA) provided results of practically the same quality as the classical 3D PSA, the calculations, however, were two to three orders of magnitude faster. The values of TPSA for all studied compounds **8aB–8iB** were 74.27 Å^2.

As suggested by Clark[17], the criterion for poor absorption of PSA>140 Å^2 appeared to be an efficient method of computationally screening large numbers of compounds. Following given, all the studied derivatives fulfilled the requirement for good absoprtion.

Palm and coworkers[18] found out that excellent correlation could be obtained between dynamic polar van der Waals´ surface areas (PSA_d) and Caco-2 permeabilities when evaluated a series of antagonists of β-adrenergic receptors (ARs). However, the major drawback of such parameter, it was computationally expensive, made it inappropriate for large database screening.

However, the RO5 violation was clearly indicated in terms of the rotatable bonds count for the compounds bearing *meta-*/*para*-propoxy or *para*-butoxy side chain, namely **8eB**, **8hB** and **8iB**, respectively. Such descriptor was a widely used filter following the finding that greater than ten rotatable bonds correlated with decreased oral bioavailability in animal models[5].

Additionally, the same derivatives have shown the predicted values of log *P* by CLOGP 4.0 approach higher than 5. Following the AARS readouts, the CLOGP 4.0 method was considered unacceptable because of providing ARRS=1.067. The main disadvantage of using such relatively unconvenient (but required, actually) fragmental-based procedure for current investigation of homological series **8aB–8iB** was that it did not take into account the position of alkoxy side chain attached to lipophilic aromatic ring – identical values were observed when *in silico* evaluated corresponding positional isomers (Table).

Similar situation was encountered when analyzing the data related to Moriguchi MLOGP. Given fragmental approach did not reflect the position of attached alkoxy side string, as expected. Surprisingly, calculated output of AARS=0.258 identified this *in silico* procedure as an acceptable tool for the prediction of log *P*. It was also documented that MLOGP>4.15 was generated for *para*-butoxy derivative (**8iB**) only.

Ghose and Crippen proposed that the qualifying range of calculated log *P* for drug-like molecules was from -0.4 to 5.6 by fragmental ALOGP procedure. The mean ALOGP was set to 2.3 and the preferred interval was 1.3–4.1, as published in[14]. Following given, propoxy- and butoxysubstituted derivatives were out of the range. In addition, the AARS for ALOGP was 0.506 so this procedure was considered disputable.

When inspecting the AARS readout assigned to miLogP 2.2, relatively conventient value was indicated (0.478). It seemed that, except for the MLOGP procedure, the miLogP 2.2 could be considered an acceptable prediction method for the *in silico* investigation of concerned compounds.

From structural viewpoint, currently studied derivatives could be also regarded as arylcarbamoyloxy-aminopropanols, i.e. a class of the compounds which act as the antagonists of β-ARs. Side effects of these drugs, which are connected with their central nervous system (CNS) activity, are well-known; for instance lethargy, depressions, psychoses[19,20] or visual hallucinations[21], respectively. Early assessment of the physicochemical properties of potentially active CNS drugs in terms of their ability to cross blood–brain barrier is extremely important.

Kelder et al.[22] previously found out that non-CNS drugs transported passively and transcellurlarly needed a PSA of 120 Å^2 or less, whereas the drugs can be targeted to the CNS with a PSA less than 60–70 Å^2. On the other hand, following the van de Waterbeemd research[23], the cutoff for PSA cutoff for CNS penetration was set to 90 Å^2 or below and molecular weight cutoff of 450. Levin suggested the molecular weight cutoff 400 or lower[24]. It was also previously documented that PSA value was dependent upon hydrogen bonding and donating atoms[25,26]. Because the hydrogen bonding was primarily associated with oxygen (O) and nitrogen (N) moieties in a molecule, then, if the sum of the N and O atoms in the structure was five or less, the compound has shown a high probability of entering the CNS. Moreover, if following difference CLOGP – (N+O) was higher than 0, then the compound had a high probability of entering the CNS.

Additionally, CNS active drugs have shown notably fewer rotatable bonds count (five or less) than other drug classes[26].

Considering the log *P* data (log P_{exp}), Hansch and Leo[27] found out that blood–brain barrier penetration was optimal when mentioned readout was within the interval of 1.5–2.7.

For the complexity of information, in paper[26] were summarized some essential attributes of successful CNS drugs. Some of them were as follows: MW<450, CLOGP<5, *n*OHNH<3, *n*ON<7, *n*rotb<8, hydrogen bonds<8 and PSA<60–70 Å^2, respectively.

Following current *in silico* calculated outputs and previously estimated data[15] as well, the compounds under the study **8aB–8iB** probably would not permeate cross blood–brain barrier so they would not probably involve the CNS side effects.

IV. Conclusion

Rather than trying to predict absorption-related quantities, researchers tried to find out general principles to distinguish drug-like from non-drug-like molecules by inspecting the drugs and non-drugs databases. Current article was focused on the *in silico* evaluation of some highly lipophilic *N*-arylpiperazine based compounds containing 2-hydroxypropan-1,3-diyl connecting chain and substituted alkoxyphenylcarbamoyloxy fragment as well, in terms of their drug-like properties. It seemed that majority of all the substances inspected successfully met the criteria which were previously pioneered especially by Christopher Lipinski in his famous Rule of Five and Daniel Veber, respectively. It could be assumed that focused compounds would potentially shown good absorption and permeation after an oral administration. In addition, following the molecular weight, PSA (TPSA), hydrogen-bond acceptors count, rotatable bonds count, CLOGP 4.0 readouts and estimated log P_{exp} data as well, it could be hypothesized that all currently inspected derivatives would not be able to cross blood–brain barrier passively and consequently not to involve CNS-related side effects.

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Botanical Standardization of the Embeli Ribes Burmf & Possibilities of Species Substitute

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Abstract- EmbeliaribesBURM. F. is an important drug of Ayurveda. Which is considered as multi remedies *with wide* group of Active consistents.Isolated from the berries.

Because of High Commerce, Traders, are subjected to 26 species of substitution, a detailed botanical investigation with macro & microscopical comparison with the drug used under the name of VIDANGA.

Therefore the present study is an attempt to establish macro & Microscopic characteristic of E.R. as well as to Distinguish the species in Chart.

GJMR-B Classification : NLMC Code: QV 35

BOTANICAL STANDARDIZATION OF THE EMBELI RIBES BURMF POSSIBILITIES OF SPECIES SUBSTITUTE

Strictly as per the compliance and regulations of:

Botanical Standardization of the Embeli Ribes Burmf & Possibilities of Species Substitute

Syed Asadulla [α], Ramandang [σ] & Rajasekharan [ρ]

EmbeliaribesBURM. F. is an important drug of Ayurveda. Which is considered as multi remedies *with wide* group of Active consistents.Isolated from the berries.

Because of High Commerce, Traders, are subjected to 26 species of substitution, a detailed botanical investigation with macro & microscopical comparison with the drug used under the name of VIDANGA.

Therefore the present study is an attempt to establish macro & Microscopic characteristic of E.R. as well as to Distinguish the species in Chart.

I. Methodology

EmbeliaribesBURM. F. Fresh aerial twigs possessing flowers and fruits was collected from I.I.H.R. Hessarghatta fields, Bangalore with accession No.1001. which is an Altitute of 800 meters (2800Sqft) latitude : 13.1323 North, longitude: 77.49332 East. Wind at East 12KM/hr, Temperature (11^0-25^0C) Humidity at 45% which is washed thoroughly in running water and the samples were deposited in institutes respository vide voucher specimen numbers 1 to 5 of sample no. 1 (FRLHT Collection No. 55181), dated January 03, 2012, to study and identified the species by Dr. Ravikumar.K. Asst. Director, RMR Division, at Institute of Ayurveda &Integreative Medicine [IAIM], an initiative of FRLHT Herbarium division.andsome of the fresh material is preserved in FAA (Formalin-Acetic acid-Alcohol) and the rest was dried at room temperature and prepared Herbarium preserved, at Research Centre & rest was dried at room temperature for Histological studies,Berry Morphology at Fig.No.1.

a) The Drug indicates identification & authentication

Great emphasis is laid on the most diagnostic characters by which each parts of the plant was identified particularly with the macro biological group to which is belongs as in Table no.1, of fruit collection and genuine and substitution (Table no. 2) which gives the value in distinguishing features between species of fruits(berries).Table no. 3 are Rare Substitution.

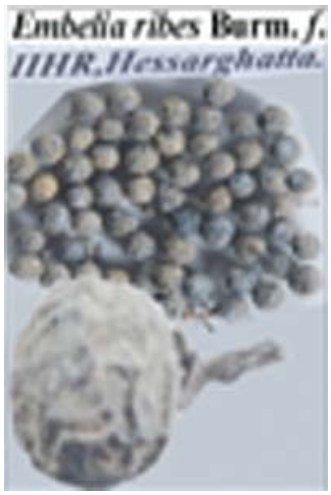

Figure 1 : (Berry)

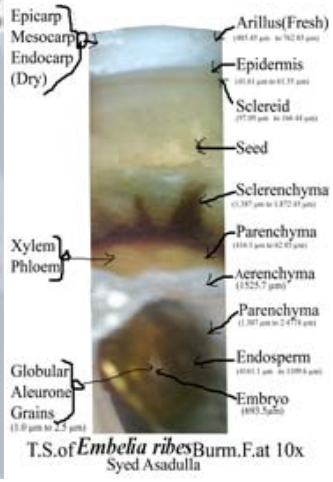

Figure 2 : (Fruit)

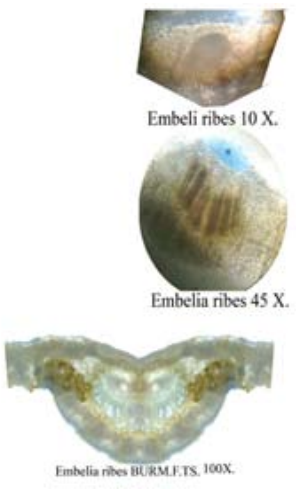

Figure 3 : (Leaf)

II. Macroscopy

MACROSCOPY of aerial portion collected with a twig with flowers & fruits. And diagnosing features are branchlets with many distinct tubercular projections leaves alternate, elliptic to oblong, base round, apex acute or shortly acuminate, papery, hairless, distinctly stalked, lateral nerves many, slender inconspicuous, with sunken glandular pits flanking lower side of the

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midrib, flowers, bisexual; minute about 2mm across, shortly stalked, white, mildly fragrant, arranged in axillary 7 terminal branched racemes, which are ca15 long. Fruit is a drupe, ca 5mm across, hairless, green with beaked apex (on ripening the fruit is dark black with distinct scaly and the details of leaf and fruit microscopy are as follows :-

III. Microscopy

Microscopy Authentic fresh leaves, fruits (berries) Fig.No.2, are collected from IIHR, B'lore, for the leaves Fig.No.3, and fruit Fig.No.2, as the Free – hand sections were taken of the aerial parts of the fresh plant to study the anatomical structure. Clearing and staining were done by the general methods.

As the leaf is first cleared in the solution of Chloral hydrate & lignification was established by the reaction with solution of phloroglucinol followed by a concentrated Hydrochloric acid (C-HCL) to detect the presence of lignin &also mounted for powder microscopy for fruit in dry condition.

The respective photographs were taken with nokiacamera and measurements were taken with camera lucida support and recorded.

IV. Anatomy

Stem - TS of fresh stem shows a circular outline, with a single layer of epidermis covered with a thin cuticle, numerous lenticels, Below the epidermis 2-5 rows of collenchymatous cortical tissue is present and rest of the cortex is parenchymatous containing numerous simple and occasionally oleoresin cells along with compound starch grains & patches of lignified fibers present at fairly regular intervals towards inner cortex, the vascular bundles, cambium, uniseriate medullary rays and pith is seen.

Petiole - TS of petiole is nearly circular in outline with a depression on the adaxial side, a thick wall epidermis, several well developed layers of collenchymas are present beneath the upper epidermis and a sheath of ground tissue, but only2 to 3 layers on Abaxial side.

Ground tissue is parenchymatous, vascular tissue forms an arc that has widely spaced bundles, a few small prismatic crystals of calcium oxalate are present in the ground tissue.

Midrib - TSFig.No.3, of leaf passing thorough the midrib shows a cuticularised upper glossy surface and lower epidermis and the midrib region shows a patch of collenchymatous cells in a depression below the upper epidermis with Prismatic crystals of calcium oxalate are present in the parenchymatous cells of the ground tissue six vascular spiral strands with thickness of 9μ to 18μ are present in the ground tissue of the midrib which is radially arranged xylem vessels intermittently phloem cells inFig.No.3.(Leaf)

Lamina - Cuticle present, upper epidermal cells in size ranges 95μ x 140 μ and the cells of the lower epidermis, ranges 120μ x 115μ in size two or three rows of palisade parenchyma cells are found below the upper epidermis with some rows of well-aerated spongy parenchyma & a few oleoresin globules are present and more numerous stomata on lower surface and with stomata on lower surfaces and matty ash grey colour with sunken glandular pits with moderate trichomes. In Fig No.4.

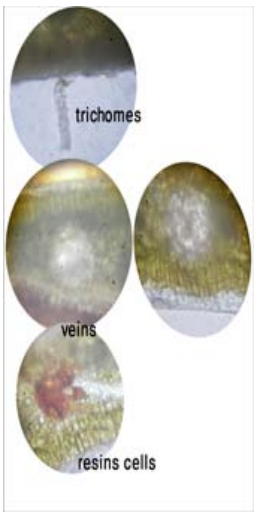

Figure 4

Fruits in fresh condition Fig.No.2.

1. In fresh condition the embryo consist of small thick parenchymatouscell and diameter size is 693.5μ with globular aleurone grains having a diameter 1.0 μ to 2.5 μ.
2. Endosperm consists of thick called parenchymatous cells layer thickness ranges from 4161.1 μ to 1109.6 μ with oil cells.
3. Perisperm:- Several layered thin walled parenchymatous cells layer thickness ranges from 208 μ to 1,248 μ along with starch grains.
4. Parenchymatous light Brown cells of lining of layer thickness ranges from 1.387 μ to 2.4774 μ.
5. Aerenchyma cells layer thickness ranges from 1525.7 μ.
6. Parenchyma 2 to 7 or several layers flattened cells layer thickness ranges from 416.1 μ to 62.85 μ with vascular bundles of xylem and phloem.
7. Sclerenchymatous dark Brown 10-14 layers which is projected intermittently radially arranged tapering ends of layer thickness ranges from 1,387 μ to 1,872.45 μ occipiedtapering ends in next abundant layers of inner parenchymatous Hexagonal cells.
8. On maturity fruits able to recognize seed structure attached with filaments and on seeds crushed in mixer show some of the somatic seeds ranges.

9. Sclereid layer of dark Brown of 2-3layers which is tangentially elongated layer thickness ranges from 97.09 μ to 166.44 μ.
10. Epidermis of 5 to 12 layers thick walled narrow and axially elongated cells layer thickness ranges from 41.61 μ to 61.35 μ.
11. In fresh condition Arilluslayer thickness ranges from 485.45 μ to 762.85 μ which is the layer of Testa is covered layer on the external side by thin transparent rectangular tangently arranged colourless cells or with collapsed parenchyma also called as membranous Arillus, In dry condition the Arillus is modified into Testa layer modified into rectangular scales 24 in number per fruit(Berries)which consist on T.S.ofepicarp, mesocarp and endocarp on powder analysis the testa region shows a group of oleoresin cells and stone cells.

V. Results and Discussions

HISTOLOGICAL Characteristics of leaf & fruit.Plays a crucial role in establishing the identity & determining the authenticity.

Detection of various anatomical features such as tracheids, trichromes, fibers, glands, cork, stomata, pollen grains etc provides important identification clues in leaf & fruits. ER and many of its species are recorded for their botanical characters in Table no.1 and 2.

EmbeliaribesBURM. F. botanical features deals with the pharmacognostical study of leaf and fruits.

VI. Discussions

EmbeliaribesBURM.F. plant character is identified in FRLHT,Bangalore, and soil samples is submitted in GKVK,Soil sciences ,, Bangalore. The results are awaiting and the Tissue culture study is carried out in I.I.H.R. Bangalore results are awaiting.

VII. Conclusion

The present statement is to predict the leaf & fruit characters of EmbeliaribesBURM. F.

Thus it is concluded that the above statement can be validated and authenticated on the basis of their macro &microscopical characters are the possibilities of substitutes.

Table 1 : Plants Seeds collected Morphological Diagnosis

Serial No.	Regions	Cortex Colour	Testa Fracture	Longitudinal Stiations or Scaly markings	Special Features
01	Hubli Hebsur	Ash Brown to green	Gradually Testais Erupted and few lines are seen.	22	Intermittent eruption in 30% of seeds
02	AyurHubli.	Brownish-Black with white patches	Testa is firmly attached	28	Few scaly eruption seen
03	Himalaya	Brown Green	Calyx, Broken, in 30%	24	Not to be seen
04	Kerala	Brownish Black	1% of Hemisphere Testa Breaks & 99% is safe	22	Nil
05	Rajastan	Brownish red to Green	Testa is erupted in 50% of seeds	22	4-5
06	Fathepur	Matte Ash – Brown	In half Hemisphere	Nil	Single Fracture
07	FRHLT	Reddish Brown	50% Testa is broken to powder	28 scales are found	Scaly depression
08	Hessarghatta	Reddish Brown to Green	Testa is attached to the seed	24 scales are found	Scaly depression
09	AmrutKesari	Brownish Black	Testa is erupted to 25%	24	2 to 3

Remarks - GenineVariety of IIHR.seeds are identified with Comparative Statement of cortex colour, Texta Fracture, Striations in the Longitudinal and Scaly Marking and Special features by which the IIHR & FRLHT Variety is genuine ,As per the Botanist Dr. Ravikumar item no 7 & 8 is E r Burm.f. and Item no 1,2,3,4,5,& 9 are embeliaTerijiumCottam and Item no 6 is not able to identify.

Table 2 : Published identified varieties

Sl. No.	Drug Name	Authour Name	Source	Uses	Published
01	EmbeliaribesBurm.f.	Chua,LSL; van Valkenburg,	JLCHForest Research Institute Malaysia, Jalan FRI, Kepong, 52109 Kuala Lumpur, Malaysia	Anthelmintic	Plant resources of South-East Asia No. 12(1): Medicinal and poisonous plants 1; de Padua, L.S., Bunyaprapatsara, N &Lemmens, R.H.M.J. (eds); Paperback edition; Bogor, PROSEA Foundation, 1999; p 257-258
01a	EmbeliaribuBurm.f.	Chua ngutVo Van Chi Tudien cay thuoc	Vietnam(grows in waste land, hill mountains)	ripped fruits Treat bitten by snack, earth worm, whites, cough and diarrhoea.	Vietnamese Medicinal Plants], Hanoi, Medicinal Publ. House, 1997; p. 244.
02	EmbeliarobustaRoxb.(Virganga. Birang-i-kabuli,)	Chua, LSL; van Valkenburg,	JLCHForest Research Institute Malaysia, Jalan FRI, Kepong, 52109 Kuala Lumpur,	berries cathartic	Plant resources of South-East Asia No. 12(1): Medicinal and poisonous plants 1; de Padua, L.S., Bunyaprapatsara, N &Lemmens, R.H.M.J. (eds); Paperback edition; Bogor, PROSEA

			Malaysia		Foundation, 1999; p 258
03	E. Basaal,	Roem&Schult	Indian	root as toothache, larger elliptical leaves, berries as forehead for pleuritis, young leaves gargle for sore throats	Trans.linn.soc.London.17:31,1834
04	Embelialacta	Tai nyuyen cay thuoc	mountains and hills	roots for treating cough and diarrhoea ,dried fruits for parasites, fever, skin-diseases;.	Sciencific and Technical Publishing House, 1993; p 221-226
05	Embelialaeta (L.) Mez	Chua nguthoatrang. Vo Van Chi Tudien cay thuoc	Vietnam	Acne Care Products	Vietnamese Medicinal Plants], Hanoi, Medicinal Publ. House, 1997; p. 245-246.
06	Embelialacta (L.) Mez. (Day)	Nguthoatrang Le QuyNguu; Tran NhuDuc Cay thuocquanh ta and Chua ngut Le Tran Duc Tronghaiva dung cay thuoc	Himalaya hills of China.	Anthelmintic.	[Popular Medicinal Plants] Hue - ThuanHoa Publishing House: 284-285 (1998) and[Planting, harvesting and usin medicinal plants] Vol. 1: 160- 170. Agr. Publishing House. Hanoi, 1984.
07	Embeliagarciniifolia),	Nguyen Dang Knoi	Vietnam	treating dysentery	National Scientific Research Centre of Vietnam Tap chi Duoc hoc [Journal of Pharmacy] (4): 11-13 (1977)
08	Embeliapicta A. DC.	Chua ngutdom Vo Van Chi Tudien cay thuoc	Vietnam	Anthelmintic.	Vietnamese Medicinal Plants], Hanoi, Medicinal Publ. House, 1997; p. 245.
09	Embeliaphilippinensis A. DC.	Rhamnuslando, Ribesoidesphilippense, Samara philippinensis), Jansen, PCM Prosea	philippinensis	Anthelmintic	Department of Plant Sciences, Wageningen Agricultural University, P.O. Box 341, 6700 AH Wageningen, Netherlands Plant resources of South-East Asia No. 13: Spices; de Guzman, C.C. &Siemonsma, J.S. (eds); Paperback edition; Bogor, PROSEA Foundation, 1999; p 253
10	EmbeliaTsjeriam Cottam.	Chua, LSL; van Valkenburg, JLCH	Malaysia	Anthelmintic	Forest Research Institute Malaysia, Jalan FRI, Kepong, 52109 Kuala Lumpur, Malaysia.

Remarks - The above nine varieties are identified and Published but the seeds are very similar in morphology to EmbeliaribesBURM.f.

Table 3 : Rare Substiuents are

Serial no	Drug name
01	Embelia Arunachal,
02	EmbeliaAustraliana
03	EmbeliaChatisghar
04	EmbeliaDasytania
05	Embeliadisticha Fletcher
06	Embeliaferruginea Wall
07	Embeliagrandifolia Fletcher
08	Embelia Gulf
09	Embelia Herbal King
10	Embelia homeopathy
11	Embelialongifolia (Benth.) Hemsl
12	Embeliamacrocarpa King & Gamble
13	Embelia Malabar
14	EmbeliaMalasian
15	EmbeliaoblongifoliaHemsl
16	EmbeliaPaciflora
17	EmbeliaParviflora
18	EmbeliapulchellaMez
19	EmbeliasessilifloraKurz
20	EmbeliastrictaCraib
21	EmbeliaVaividangam
22	Embeliavillosa Wall

Remarks - The above Twenty two varieties are identified but not published but the seeds are very similar in morphology to EmbeliaribesBURM.f.

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Pharmacological Activity and Chemical Constituents of Eclipta Alba

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Abstract- Eclipta alba is a herb commonly found throughout India. This plant is known to have various pharmacological activities and is traditionally used in treatment but it lacks adequate scientific proof of this activity and constituents responsible for it. The present paper describes the phytochemical and pharma-cological investigations of Eclipta alba.

GJMR-B Classification : NLMC Code: WB 330

Strictly as per the compliance and regulations of:

Pharmacological Activity and Chemical Constituents of Eclipta Alba

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Abstract- ***Eclipta alba*** **is a herb commonly found throughout India. This plant is known to have various pharmacological activities and is traditionally used in treatment but it lacks adequate scientific proof of this activity and constituents responsible for it. The present paper describes the phytochemical and pharma-cological investigations of** ***Eclipta alba.***

I. Introduction

Family : Asteraceae *Eclipta Alba* (Asteraceae) is an annual herbaceous plant, commonly known as false daisy. It is an erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate. It is also known as Bhringaraj and Karisilakanni, which is found a common weed throughout India ascending up to 6000 ft. The specific Eclipta Alba means white which refers to the colour of the flowers. Main active principles consist of coumestans like wedelolactone, desme-thylwedelolactone (Wagner H. *et al.*, 1986), furanocoumarins, oleanane & taraxastane glycosides (Amritpal Singh.*et al.*, 2010).

Ethnopharmacology : Eclipta Alba (L.) has been used in various parts of tropical and sub-tropical regions like south America, Asia, Africa. There are three kinds or *Eclipta Alba*-the white-flowering, the yellow-flowering, and the black-fruiting, but all three grow throughout India by marshes, rivers, and lakes or on the foothills of the Himalayas. It is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation. It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases (Scott Treadway1998). The alcoholic extract of the plant has shown antiviral activity against Ranikhet disease virus (Sunita Dalal, *et al.*, 2010). The plant is commonly used in hair oil all over India for healthy black and long hair (Roy RK, *et al.*, 2008). The fresh juice of leaves is used for increasing appetite, improving digestion (Cheryl Lans, 2007) and as a mild bowel regulator. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma and popularly used to enhance memory and learning (Otilia Banji, *et al.*, 2007).

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The plant has a reputation as an antiageing agent in Ayurveda (Thakur VD, *et al.*, 2005). It is used as a general tonic for debility. Externally it is used for inflammation (Amritpal singh, et al., 2008; and Mahesh Sawant, *et al.*, 2004), minor cuts and burns and the fresh leaf-juice is considered very effective in stopping bleeding (Mukherjee DR, *et al.*, 1976). Leaf juice mixed with honey is also used for children with upper respiratory infections and also used in eye and ear infections. It is a source of coumestans-type compounds used in phytopharmaceutical formulations of medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis (Wagner H. *et al.*, 1986).

Figure : Eclipta Alba with White flower

It is widely used in India as a chologuague and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder (Scott Treadway, 1998). The water extract of *Eclipta prostrata* (whole plant) exhibited the most potent inhibitory activity against HIV-1 integrase (HIV-1 IN) (Tewtrakul S, *et al.*, 2007). Vedic Guard, a polyherbal formulation is a synergistic combination of 16 medicinal plant extracts contains *Eclipta Alba* as a major ingredient (Rema Razdan, et *al.*, 2008). Charaka advises taking the juice of *Eclipta Alba* with honey to prevent the onset of senility, and its oil as the best medicated massage oils for rejuvenation therapies.

Phytochemistry: Eclipta Alba (L.) contains wide range of active principles which includes coumestans, alkaloids, flavonoids, glycosides, polyacetylenes, tri-terpenoids. The leaves contain stigmasterol, β-terthienyl-methanol, wedelolactone, demethylw-edelolactone and demethylwedelolactone-7-glucoside (Wagner H. *et al.*,

1986).The roots give hentriacontanol and heptacosanol. The roots contain polyacetylene substituted thiophenes.The aerial part is reported to contain a phytosterol, β-amyrin in the n-hexane extract and luteolin-7-glucoside, β- glucoside of phytosterol, a glucoside of a triterpenic acid and wedelolactone in polar solvent extract (Jadhav VM, *et al*., 2009). The polypeptides isolated from the plant yield cystine, glutamic acid, phenyl alanine, tyrosine and methionine on hydrolysis. Nicotine and nicotinic acid are reported to occur in this plant (Jadhav VM, *et al*., 2009).

Coumestan: Coumestan is an organic compound that is a derivative of coumarin. Coumestan forms the central core of a variety of natural compounds known collectively as coumestans. Coumestans, including a coumestrol and phytoestrogen are found in a variety of plants. Because of the estrogenic activity of some coumestans, a variety of syntheses have been developed that allow the preparation of coumestans so that their pharmacological effects can be explored. The major coumestan isolated from Eclipta Alba includes wedelolactone 0.5-0.55% and desmethylwedelolactone (Neerja Kaushik-Basu, *et al*., 2008).

Terpenoids and their glycosides: Taraxastane triterpene glycosides, named eclalbasaponins VII-X were isolated, along with four oleanane glycosides eclalbasaponins I-VI. The structures of eclalbasaponins VII-X were characterized as 3β, 20β, 16β and 3β, 20β, 28β trihydroxytaraxastane glycosides, and their sulphated saponins (Shoji yahara, *et al*., 1997). Two oleanane-type glycosides eclalbasaponin I and eclalbasaponin II along with the ubiquitous steroid, stigmasterol were isolated from an n-hexane extract of the stem bark of *Eclipta prostrata* (Mohammad S et al., 2005). From the whole part of *Eclipta Alba* Hassk., six new triterpene glycosides, named eclalbosaponins I-VI, were isolated these structures were characterized as echinocystic acid glycosides and those of V –VI were revealed to be sulphated saponins (Shoji yahara, *et al*.,1994).

Alkaloids: The clinical tests showed that the herb contains the alkaloid ecliptine. Bioassay-guided fractionation of the MeOH extract of *Eclipta Alba* using three yeast strains (1138, 1140, and 1353) resulted in the isolation of eight bioactive steroidal alkaloids (1–8), six of which are reported for the first time from nature. The major alkaloid was identified as (20S)(25S)-22,26-imino-cholesta-5,22(N)-dien-3β-ol (verazine, 3), while the new alkaloids were identified as 20-epi-3-dehydroxy-3-oxo-5,6-dihydro- 4,5-dehydroverazine (1), ecliptalbine [(20R)-20-pyridyl-cholesta-5-ene-3β,23-diol] (4), (20R)-4β-hydroxyverazine (5), 4β-hydroxyverazine (6), (20R)-25β-hydroxyverazine (7), and 25β-hydroxyverazine (8). Ecliptalbine (4), in which the 22, 26-imino ring of verazine was replaced by a 3-hydroxypyridine moiety, had comparable bioactivity to verazine (Maged S. *et al*., 1998).

Volatile oils: The volatile components were isolated from the aerial parts of this plant by hydrodistillation and analysed by GC–MS. A total of 55 compounds, which were the major part (91.7%) of the volatiles, were identified by matching mass spectra with a mass spectrum library (NIST 05.L) (Xiong-HaoLin,*et al*., 2010).

Saponins: From the whole plant of *Eclipta Alba,* a new triterpene saponin, named eclalbatin, together with alpha-amyrin, ursolic acid and oleanolic acid were isolated. Dasyscyphin C was isolated from *Eclipta prostrate* which were studied on the HeLa cells for the anticancer activity (Khanna, et al., 2008).

Bioactivity: Eclipta Alba is a plant used in folk & traditional medicine for cirrohosis and infectious diseases1. It is believed to prevent aging and rejuvenate hair, teeth, bone, memory, sight, hearing. The plant was known to possess significant antifungal and insecticidal properties. The biological properties of the plant are treated under two subheadings: (i) pharmacological properties (ii) insecticidal properties and other biological properties.

Table 1 : Parts containing chemical constituents of *Eclipta Alba*

S.No.	Parts	Chemical constituents
1	Leaves	Wedelolactone[1.6%], Desmethylwedelolactone, Desmethyl-wedelolactone-7-glucoside, stigmasterol
2	Roots	Hentriacontanol, Heptacosanol & Stigmasterol, Ecliptal, Eclalbatin.
3	Aerial parts	β-amyrin & Luteolin-7-0-glucoside, Apigenin, Cinnaroside, Sulphur compounds, Eclalbasaponins I-VI
4	Stems	Wedelolactone
5	Seeds	Sterols, Ecliptalbine (alkaloid)
6	Whole plant	Resin, Ecliptine, Reducing sugar, Nicotine, Stigmasterol, Triterpene saponin, Eclalbatin,Ursolic acid, Oleanolic acid.

II. Pharmacological Properties

Crude extract : The crude extract has wound healing properties. The loss of hepatic lysosomal acid phosphatase and alkaline phosphatase by CCl4 was significantly restored by *Eclipta Alba.* The previous studies shows that hepatoprotective activity of Eclipta Alba is by regulating the levels of hepatic microsomal drug metabolizing enzymes (Saxena AK, *et al*., 1993). The fresh plant is used as self medication by AIDS

patients in southern Thailand and showed potential as a therapeutic agent against Giardia intestinalis infections (Sawangjaroen N, *et al.,* 2005 and Supinya Tewtrakul, et al., 2006). The leaf extract showed hypolipedemic activity in atherogenic diet induced hyperlipedemic rats (Dhandapani R. 2007). It has antimicrobial and antioxidant properties (Karthikumar S, *et al.,* 2007). 3% extract of Eclipta Alba is used in pilex formulation with other ingredients. It has been reported to decrease bleeding time (Mukherjee DR, *et al*., 1976). Leaf extract has been used in edema. It is used in the treatment of paronychia (Abdul viqar khan, *et al*., 2008).

Table 2 : Pharmacological activities of the chemical constituents of *Eclipta Alba*

Sl.No	Chemical constituents	Pharmacological activites
1	Wedelolactone	Antihepatotoxic (Nazim Uddin, *et al.,* 2010), Antibacterial (Karthikumar S, *et al.,* 2007), Trypsin Inhibitor, Antivenom (Vianna-da-silva NM, *et al.,* 2003)
2	Eclalbosaponins	hair revitalizing (Rupali thorat, *et al.,* 2009), Antiproleferative (Khanna, *et al.,* 2008 and Neerja Kaushik-Basu, *et al.,* 2008), Antigiardial (Sawangjaroen N, *et al.,* 2005)
3	Demethylwedelolactone	Antihepatotoxic (Wagner H. *et al.* 1986), Antihaemorrhage (Mukherjee DR, *et al*., 1976), Antivenom (Vianna-da-silva NM, *et al.,* 2003), Dye (cosmetic) (Meena AK, *et al.,* 2010)
4	Dasyscyphin C	Antiviral, Anticancer (Khanna, *et al*., 2008)
5	Eclalbatin	Antioxidant(Tewtrakul S, *et al.,* 2007)
6	Ecliptalbine	Verazine Lipid lowering, Analgesic(Maged S. Abdel-Kader *et al*., 1998)

Anti – Ulcer Activity : Eclipta Alba has the anti-ulcer activity. There are different type of herbal plants which are used to treat anti-ulcer such as The Polyherbal Formulation – RO7D consists of eleven medicinal plants namely Centella asiatica, Cassia auriculata, Cynadon dactylon, Rosa damascene, Myristica fragrans, Nelumbo nucifera, Hibiscus rosa-sinensis, Hemidesmus indicus, Glycyrrhiza glabra, Eclipta alba and Phyllanthus niruri. The Polyherbal Formulation – RO7D exhibited ($P<0.001$) significant decrease in ulcer index in both the model and significant decrease in the gastric volume in pyloric ligation rat ulcer model. The study indicates that extract RO7D has anti-ulcer activity and its anti-ulcer potential may be due to anti –secretary and cyto-protective activity. (Srinivasan D et al., 2008)

Immunomodulator activity : The protection of neuronal tissue may be possible due to the immunomodulatory action of *Eclipta Alba.* Due to methanol extract of whole plant of *Eclipta Alba* (1.6% wedelolactone), the phagocytic index antibody titer, phagocytic index and WBC count increased. Due to inhibition of non-specific humoral (lysozyme, antipr-otease and complement) and cellular (myelop-eroxidase content), the reactive oxygen and nitrogen species are produced.The dietary intake of *Eclipta Alba* aqueous leaf extract also enhance the non-specific immune response and disease resistance of o.massambicus against A.Hydrpphila. *Eclipta Alba* serve as a potential memory modulator also (Ghosh M *et al.,* 1984 And Roitt I *et al.,* 1998, Hudson L *et al.,* 1991).

Hair growth and alopecia : Eclipta Alba is a well known Ayurvedic herb for hair growth. *Eclipta Alba* is used in hair oil preparation since it promotes hair growth and maintains hair black.10%w/v of *Eclipta Alba* used as a main ingredient in the preparation of herbal formulation for hair growth. Petroleum ether and ethanolic extract were also used in oleaginous cream and applied topically (Roy R, *et al.,* 2008).

Anticancer activity : The methanolic extract of Eclipta Alba has the inhibitory effect against colon cancer due to inhibition of proliferation of cancer cells in a concentration dependent manner (Ruddon R.W, *et al.,* 1995 and St. Luke, *et al.,* 2007). Methanolic extract of Eclipta Alba was evaluated for its anticancer activity against Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice. On day 1, the extract of Eclipta Alba at a dose of 250 and 500 mg/kg body weight were administered orally and continued for 9 consecutive days. The anticancer activity was examined by determining the tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span in experimental animal models. The extract increased the life span of EAC treated mice and restored the hematological parameters as compared with the EAC bearing mice. Thus, study revealed that the methanolic extract of Eclipta Alba showed anticancer activity in the tested animal models (Malaya Gupta, et al., 2005). Coumestans are also known to act as phytoestrogens. These compounds are present in soyabeans and clover. In many countries it is used as diet which act as chemopreventive agent in breast and prostate cancer (Neerja Kaushik-Basu, et al., 2008). Dasyscyphin-C (saponins) a newer isolated compound from Eclipta prostrata reported to have anticancer-cytotoxic activity. It was tested under invitro conditions in HeLa (Human cervical carcinoma) & vero cell lines. At the concentration of 50μg/ml it showed a good anticancer-cytotoxic activity on HeLa cells (Khanna, 2008). A rat hepatic stellate cell line (HSCs) was used as in-vitro assay system, the methanolic extract of aerial parts of Eclipta prostrata showed significant inhibitory activity on HSCs proliferation (Mi Kyeong Lee, *et al.,* 2008).

Hepatoprotective effect : Eclipta Alba is considered a powerful liver tonic. The hepatoprotective potential of *Eclipta Alba* was studied by assessing the biochemical parameters like lipid peroxide (LPO), superoxide dismutase (SOD), Catalase (CAT),

glutathione peroxide (GPx), glutathione reductase (GR), ascorbic acid and α-tocopherol. Oral administration of the Eclipta Alba significantly decreased levels of LPO and elevated the activity of antioxidant enzymes SOD, CAT, GPx, and GR as well as endogenous levels of ascorbic acid and α-tocopherol. Eclipta Alba has show protective effect on experimental liver damage in rats and mice and also used for the treatment of liver cirrhosis and infective hepatitis by reducing centrilobular necrosis, hydropic degeneration and fatty change of the hepatic parenchymal cells (Singh B *et al.,* 1993*)*. The coumestans constituents of the *Eclipta Alba* plant, wedelolactone and demethylwedelolactone, are responsible for the potent antihepatotoxic activities in CCL_4 – galactosamine and phalloidin induced liver damage in rats (Chopra R *et al.,* 1996). The ethyl acetate fraction of Eclipta Alba improves the both enzymatic and non enzymatic antioxidant status in rat liver (Singh B *et al.,* 1993). Hepatoprotective activity also expressed by *Eclipta Alba* by regulating hepatic lysosomal enzymes (Saxena A, *et al.,* 1993). Hepatoprotective activity of methanolic extract and sub fractions of leaves and the chloroform extract and sub fractions of roots of *Eclipta alba* was carried out using carbon tetrachloride- induced liver damage and Lysosomal enzymes level in wistar albino rats. The methanolic extract of leaves and the chloroform extract of roots of Eclipta Alba showed significant activities and respectively causing 72.8% & 47.96% reduction of lysosomal enzyme. The triterpenoid eclabasaponin fraction from methanolic extract of leaves produced significant (78.78%) and the alkaloidal fraction (60.65%) reduction of carbon tetra chloride induced increase in lysosomal enzyme in blood. Coumestan fraction and triterpenoidal saponin fraction from the chloroform extract of roots produced very significant (75.6%) and (52.41%) respectively reduction of carbon tetra chloride induced increase in lysosomal enzyme levels in blood (Lal V.K, et al., 2010).

Anti-inflammatory and Analgesic activity : The extract of *Eclipta Alba* was administered orally to investigate anti-inflammatory activity (Amritpal singh, *et al.,* 2008). The anti-inflammatory activity which estimated by using carragenan induced paw oedema model.Inflammation occures due to activation of platelet activation factors and release of pro-inflammatory mediators such as prostaglandins, kinins, tumor necrosis factors and nitric acid. The extract of *Eclipta Alba* has the potent inhibitor of the pro-inflammatory transcription factors and a promising agent for the treatment of the inflammatory cascade of cardio-vascular diseases (Kaileh *et al.,* 2007).

Antidiabetic Activity : Extracts of various plant materials capable of decreasing blood sugar (Bopanna K *et al.,* 1982). The chloroform extract of *Eclipta Alba* exhibited significant anitidiabetic activity in alloxan induced diabetic rats. This extract has showed improvement in parameters like body weight and lipid profile by enhancing antioxidant defenses to protect against oxidative damage (Hodges, C *et al.,* 1989 and Nahar N *et al.,* 1993). The active principles present in *Eclipta Alba* has been reported to possess pancreatic beta cells regenerating, insulin releasing and fighting the problem of insulin resistance (Welihinda J *et al.,* 1982).

Antihyperlipedemic properties : It has been reported that in the atherogenic diet induced hyperlipedemic model, the aqueous leaf extract of the *Eclipta prostrata* was given orally to the rats which significantly reduced total cholesterol, triglycerides, total protein. There was a significant elevation in the high density lipoprotein cholesterol levels (Dae-Ik Kima, *et al.*, 2008).

Antioxidant properties: The antioxidant effects of *Eclipta prostrata* was reported when the level of serum hydroxyl radical (nmol/mg protein per minute) and serum lipid peroxide (nmol/mg protein) levels reduced as compared to untreated group. 100mg/kg dose significantly reduced Carbonyl content of oxidatively modified proteins8. Antioxidant activity of Eclipta prostrata was determined by FRAP, radical scavenging activity, reducing activity, and DPPH assay. The antioxidant capacity was increased by increasing the concentration of the extracts from 25 to 100mg/ml27. The antioxidant activity of the hexane, ethyl acetate, ethanol and water extracts of E. prostrata was determined by ferric thiocynate (FTC) (Karthikumar S, *et al.*, 2007).

Anthelmintic activity: The methanolic extract of whole plant of Eclipta Alba (L.) have the anthelmintic activity.

Other pharmacological activities: It has been reported that the importance of free carboxylic acid at C-28 position in echinocystic acid derivatives from the methanolic extract Eclipta prostrata showed antifibrotic activity (Mi Kyeong Lee, *et al.*, 2008). Ethanolic and ethyl acetate fractions of Eclipta prostrata were tested for its antibacterial activities against Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus (Karthikumar S, *et al.*, 2007). Eclipta prostrata is combined with a non-plant material which is used to bath children suffering from malnutrition for 9 days and used as self medication by AIDS patients in southern Thailand (Sawangjaroen N, *et al.*, 2005 and Cheryl Lans. 2007). 16 parts of Eclipta prostrata (bhringaraj), 1 part of Triphala formula {Emblica officinalis (amalaki), Terminalia chebula, (haritaki), Terminalia belerica (bibhitaki)}, 1 part of Caltropis gigantean (arka) and 1 part of Smilax officinalis (sariva) mixed with 80 parts of sesame oil and boiled to make a medicated oil which is reported to be used in skin diseases (Bensky Dan, *et al.*, 1986).

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In Vitro Antidiabetic Activity of *Cardiospermum Halicacabum* leaves Extracts

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Abstract- *Objective:* The present study was designed to investigate the glucose uptake of (antidiabetic activity) crude n-hexane,ethanol, methanol and aqueous leaf extracts of Cardiospermum Halicacabum.

Methods: of Cardiospermum Halicacabumleaf extracts were subjected to inhibitory effect of glucose utilization using specific standard in vitro procedure.

Results: results in four different leaf extracts revealed that, the methanol extract at a concentration of 50g plant extract/l was found to be more potent than other extracts with the lowest mean glucose concentration of 201±1.69 mg/dl at the end of 27 hrs.

Conclusions: The present findings suggest that, the methanolic extract showed a significant inhibitory effect on glucose diffusion in vitro thus validating the traditional claim of the plant.

Keywords: *cardiospermum halicacabum, antidiabetic activity, glucose diffusion method.*

GJMR-B Classification : *NLMC Code: QV 4*

Strictly as per the compliance and regulations of:

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Stalin.C [α], Vivekanandan.K [σ] & Bhavya.E [ρ]

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I. Introduction

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both[1]. According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3% [2]. Management of diabetes without any side effect is still a challenge to the medical community. The use of the drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects[3]. Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs[4].

The plant *Cardiospermum halicacabum Linn.* (Sapindaceae) is an annual or sometimes perennialclimber, commonly found as a weed throughoutIndia. The tender, young shoots are used as avegetable, fodder, diuretic, stomachic, andrubefacient[5,6]. It is used in rheumatism, lumbago,nervous diseases, and as a demulcent in orchitis andin dropsy. In Sri Lanka, it is used for the treatmentof skeletal fractures. The juice of the herb is used tocure ear-ache and to reduce hardened tumours[7]. Itexhibits significant analgesic, anti-inflammatoryand vaso-depressant activity, which is transient in nature. *In vitro* studies have revealed itsantispasmodic and curative actions confirming theuse of the herb in Ayurvedicmedicine[8]. The leaves of this plant mixed with castor oilare administered internally to treat rheumatism andto check lumbago[9].The present investigation is directed to the exploration of the antidiabetic activity based on the study of the various extracts of *Cardiospermum halicacabum*which show inhibitory effect of glucose utilization and, are in use as hypoglycemic agent in traditional system of medicine.

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II. Materials and Methods

a) Plant material

The fresh plants of*Cardiospermum halicacabum* were collected from Nellore (AndhraPradesh) and authenticated by Dr.P.Jayaraman, Ph.D., Director, Plant Anatomy Research Centre, Medicinal Plants Research Unit, Tambaram, Chennai-45. A portion of the sample was kept in the department museum for further reference (PARC/2010/579).

b) Preparation of extracts

The shade dried powdered form of leaves of *Cardiospermum halicacabum*was taken and subjected to successive extraction using n-hexane, Ethanol, and methanol by continuous percolation process in soxhlet apparatus. The aqueous extract was prepared by the maceration with double distilled water. Each extract was concentrated by distilling off the solvent and evaporated to dryness. The extracts were dissolved in 1% carboxy methyl cellulose (CMC) and used for the present study.

c) Effects of Various Extracts on In vitro Inhibitory Glucose Diffusion

A simple model system was used to evaluate the effects of Cardiospermum halicacabum leaf extracts on glucose movement in vitro. The model was adapted from a method described by Edwards et al.[10] which involved the use of a sealed dialysis tube into which 15ml of a solution of glucose and sodium chloride (0.15M) was introduced and the appearance of glucose in the external solution was measured. The model used in the present experiment consisted of a dialysis tube (6cmX15mm) into which 1ml of 50g/litre plant extract in 1% CMC and 1ml of 0.15M sodium chloride containing 0.22M D-glucose was added. The dialysis tube was sealed at each end placed in a 50ml centrifuge tube containing 45ml of 0.15M sodium chloride. The tubes were placed on an orbital shaker and kept at room

temperature. The movement of glucose into the external solution was monitored at set time intervals.

d) Statistical Analysis

Data are expressed as mean ± S.E.M. Statistical comparisons between groups were done by one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison tests to analyze the differences. $p<0.001$ were considered as significant.

III. Results

a) Effect on Glucose Diffusion

With the distinctive traditional medical opinions and natural medicines mainly originated in herbs, traditional medicine offers good clinical opportunities and shows a bright future in the therapy of diabetes mellitus and its complications. The effect of *Cardiospermum halicacabum* leaves as anti-diabetic agents has been studied. All extracts showed varying effect on glucose utilization. These extracts caused a significant decrease in glucose concentration during the experiment. The effects of *Cardiospermum halicacabum* leaves extracts on glucose diffusion inhibition were summarized in Table.1. At the end of 27 hrs, glucose movement of control (without plant extract) in the external solution had reached a plateau with a mean glucose concentration above 300mg/dl (314±2.89). It was evident from the table that the methanol and aqueous extracts were found to be potent inhibitors of glucose diffusion ($p<0.001$) compared to control. The methanol extract was found to be more potent than other extracts showing the lowest mean glucose concentration of 201±1.69 mg/dl at the end of 27 hrs (Table.1)

Table 1 : Effect of *Cardiospermumhalicacabum* leaves extracts (50g/litre) on the movement of glucose out of dialysis tube over 27hr incubation period

Extract	1h	3h	5h	24h	27h
Control(in the absence of extract)	133.13±1.13	212.13±2.23	232.13±1.56	311.15±1.85	316.2±2.89
n-Hexane extract (50g/l)	108.36±2.18***	165±1.91***	210.12±1.16***	263.11±1.84***	301.26±1.86***
Ethanol extract (50g/l)	97.17±1.91***	156±0.33***	189.55±0.68***	246.12±2.65***	260±1.62***
Methanol extract (50g/l)	76.22±0.36***	102±1.84***	140.59±1.30***	198±1.36***	201±1.69***
Aqueous extract (50g/l)	80.62±0.72***	113.15±0.31***	145.21±2.21***	201.15±2.22***	213.11±1.44**

Values are expressed as mean ± SEM of triplicate; Data were analysed using one way ANOVA followed by Tukey-Kramer multiple comparison test; ***$P<0.001$ compared to control.

IV. Discussion

Diabetes mellitus is a debilitating and often life threatening disorder with increasing incidence throughout the world. There is a steady rise in the rate of incidence of Diabetes mellitus and estimated that 1 in 5 may be diabetic by 2025 [11]. Antihyperglycemic activities of most effective plants were in part explained by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and/or to stimulate insulin secretion [12]. In the present study, research has been carried out to evaluate the potential of various extracts to additionally retard the diffusion and movement of glucose in the intestinal tract [13].

A decoction of *Cardiospermum halicacabum* leaves is used worldwide for the treatment of various ailments including antidiabetic. The numerous polyphenolic compounds, triterpenoids and other chemical compounds present in the plant may account for the observed antidiabetic effects of the leaf extracts. A Decoction of *Cardiospermum halicacabum* leaves was screened for hypoglycaemic activity on alloxan-induced diabetic rats. In both acute and sub-acute tests, the water extract, at an oral dose of 250 mg/kg, showed statistically significant hypoglycaemic activity[14]. The treatment with *Cardiospermum halicacabum* aqueous leaf extract (0.01-0.625 mg/mL) showed significant inhibition on LDL glycation in a dose-dependent manner. Tannins, flavonoids, apigenin, pinitol and luteolin, and other chemical compounds present in the plant are speculated to account for the observed hypoglycaemic and hypotensive effects of the leaf extract.

V. Conclusion

The present study demonstrates the ability of various extracts of *Cardiospermum halicacabum* to inhibit glucose diffusion using an *in vitro* model of glucose absorption. In particular, methanol and aqueous extracts represent potential inhibitory of glucose diffusion supplements that may be useful for allowing flexibility in meal planning in type II diabetes. Further studies are required to elucidate whether in vitro effects represent therapeutic potential by limiting postprandial glucose absorptions and for improving glycemic control in type 2 diabetic subjects.

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Synthesis and Evaluation of Quinazolinone Derivatives for Cardiovascular Activity

By Navneet Singh, R C Agarwal & C P Singh
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Abstract- Synthesis and evaluation of 3-(p methoxybenzylidene) hydrazinoacetylamino-2-methyl-6- bromoquinazolin-4-(3H)-one and 3-(p-N, N-dimethylbenzylidenylamino)hydrazine-acetylamino-2-methyi-quina-zolin-4 (3H)-one for cardiovascular activity.

Synthesis and evaluation of 3-(p methoxybenzylidene) hydrazinoacetylamino-2-methyl-6-bromoquinazolin-4-(3H)-one and 3-(p-N, N-dimethylbenzylidenylamino)hydrazine-acetylamino-2-methyi-quina-zolin-4 (3H)-one for cardiovascular activity methy-lmono substituted quinazolin-4(3H)-onyl)]-5'-(sub-stitu-tedphenyl)-tetrazolinum chloride (compounds 25-34).

Keywords: quinazolinone derivatives, formazan, hydrazi-noacetylamino, cardiovascular, antihypertensive activity.

GJMR-B Classification : NLMC Code: WC 168

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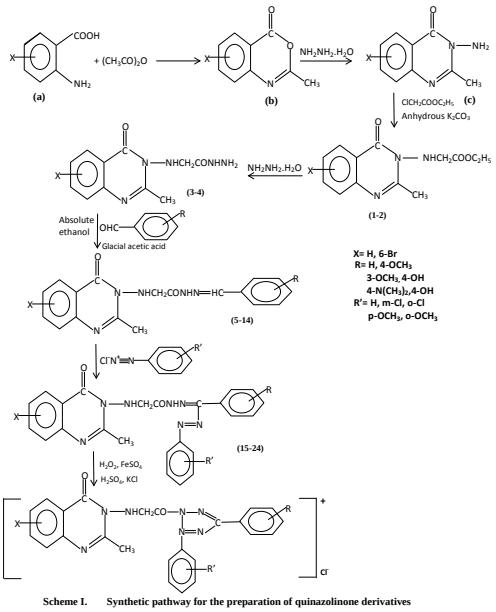
Synthesis and Evaluation of Quinazolinone Derivatives for Cardiovascular Activity

Navneet Singh [α], R C Agarwal [σ] & C P Singh [ρ]

Graphical Abstract- Synthesis and evaluation of 3-(p methoxybenzylidene) hydrazinoacetylamino-2-methyl-6-bromoquinazolin-4-(3H)-one and 3-(p-N, N-dimethylbenzylidenylamino)hydrazine-acetylamino-2-methyi-quina-zolin-4 (3H)-one for cardiovascular activity.

SCHEME I



Scheme I. Synthetic pathway for the preparation of quinazolinone derivatives

Abstract- 3-(ethylacetylamino)-2-methylmono substitutedquinazolin-4(3H)-ones (compounds 1-2) were prepared by the reaction of ethylchloroacetate with 3-amino-2-methylmono-substituted quinazolin-4(3H)-one in dry acetone in the presence of anhydrous K_2CO_3. Compounds 1-2, on reaction with hydrazine hydrate (99-100%) in methanol gave 3-(hydrazinoacetylamino) -2-methylmono substitutedquinazolin-4 (3H) -ones (comp-ounds 3-4), which on reaction with different aromatic aldehyde gave 3- (substitutedarylidene) hydrazine-oacetylamino-2-methylmono substitutedquinazolin-4 (3H) -ones (compounds 5-14). These compounds (5-14) on reaction with substituted benzene diazonium chloride yielded 3-[(acetylamino-2-methylmono substitutedquinazol in-4(3H)-onyl)]-1'-(substitutedphenyl)-3'- (substitutedaryl)- formazans (compounds 15-24). These compounds on oxidation with H_2O_2, ferrous sulphate and H_2SO_4 showed intramolecular cyclization to give- 2' (substitutedaryl) 4'-[3-(acetylamino)-2-methyl-mono substituted quinazolin4(3H)-onyl)]-5'-(sub-stitutedphenyl)-tetrazolinum chloride (compounds 25-34).

Keywords: quinazolinone derivatives, formazan, hydrazinoacetylamino, cardiovascular, antihypertensive activity.

I. Introduction

Quinazolin-4-(3H)-one, a potent pharmacodynamic heterocyclic nucleus has gained prominence in medicinal chemistry because it possess a wide spectrum of biological activities i.e. anticonvulsant [1], antibacterial [2], anti-inflammatory [3], antimicrobial [4] as well as antiproliferative [5]. Substitution at 2/3-position of quinazolinone ring imparts the cardiovascular activity [6-7], antitubercular activity [8], CNS depressant [9], antifungal [10], anticancer [11], analgesic [12] and antihypertensive [13]. Considering quinazolinone nucleus as potent pharmacophore for the cardiovascular activity, some newer derivatives of quinazolinone had been synthesized (Scheme I). The purity of the compounds was checked by TLC using silica gel G. The structure of all the compounds was confirmed by analytical and spectral data. All the newly synthesized compounds were screened for the elemental analysis (Table I) and their cardiovascular activity (Table II). The most active compounds of this series were 6 and 13. The ALD_{50} of these compounds were >2000 mg/kg p.o., indicating good safety margin.

II. Pharmacological Result and Discussion

Compounds 5, 7, 8 and 9 elicited potent immediate fall of varying degree (20-40 mmHg) and delayed fall of varying degree (10-40 mmHg) and duration (45-60 minutes) (Table II). Compounds 5 and 7 were associated with inhibition of CO and NA responses. Such a cardiovascular profile is suggestive of peripheral site of action. Compounds 8 and 9 were associated with inhibition of CO without affecting the NA response which might be suggestive of central site of action. In addition these compounds 8 and 9 showed increase in HR (tachycardia) of 1-2 beats per minutes and 3 bpm respectively. Compound 6 i.e. 3-(p-methoxybenzylidene) hydrazinoacetylamino-2-methyl-6-bromoquinazolin-4 (3H)-one showed an immediate fall in blood pressure (35 mmHg) followed by potent and gradual fall in blood pressure (70mmHg) as compared to the control value. The blood pressure lowering activity

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of this compound lasted for 95 minutes at a dose of 2.5 mg/kg i.v. In addition, this compound inhibited both pressor responses without affecting resting HR, which might be suggestive of peripheral site of action of this compound. As this compound had shown potent cardiovascular activity, it was studied at three graded doses (1.25, 2.5 and 5 mg/kg i.v.). The cardiovascular results are given in table II. Compounds 10, 11, 12 and 14 had also exhibited the promising hypotensive activity of varying degree (30-50 mmHg) and duration (60-75 minutes). In addition these compounds were associated with inhibition of CO without affecting the NA response, which might be suggestive of central site of action of these compounds. The most active compound of this series was 13 i.e. 3-(p-N,N-dimethylbenzylidenylamino)-hydrazinoacetylamino-2-methyl-quinazolin-4(3H)-one. Considering its potentiality it was further studied at 3 graded doses (1.25, 2.5 and 5 mg/kg i.v.). In lower doses 1.25 and 2.5 mg/kg i.v. it showed an immediate fall in blood pressure (35 mmHg and 52 mmHg) and delayed fall (70 mmHg and 100 mmHg) respectively. The hypotensive activity of this compound lasted for 100 and 130 minutes respectively. In higher doses i.e. 5 mg/kg i.v. it showed a very potent hypotensive activity i.e. an immediate fall (80 mmHg) and delayed fall (130 mmHg), which lasted for 195 minutes. Compound 13 was associated with inhibition or abolition of CO response without affecting the NA and heart rate. Such a pharmacological profile might be suggestive central site by action of this compound. The diazotized products i.e. compounds (15-24) showed moderate to potent hypotensive activity of varying degree (35-70 mmHg) and short duration (20-40 minutes). These compounds did not affect the heart rate and pressor responses (CO and NA). Moreover, the conversion of these compounds into their corresponding 5- member tetrazolinum salts resulted in compounds (25-34) having mild hypotensive activity (5-20 mmHg) of short duration (5-15 minutes). They appear to be acting directly on the smooth muscles of blood vessels (direct vasodilators) because these compounds did not affect the CO and NA responses and had short duration of action.

III. Conclusion

The intensive study of the cardiovascular profile of the synthesized compounds suggested that compounds 6 and 13 i.e. 3-(p-methoxybenzylidene) hydrazinoacetylamino-2-methyl-6-bromoquinazolin-4 (3H)-one and 3-(p-N, N-dimethylbenzylidenylamino)-hydrazinoacetylamino-2-methyl-quinazolin-4(3H)-one respectively had excellent cardiovascular activity. Therefore, these compounds should attract the interest of researchers and pharmaceutical companies for clinical studies and other applications in the therapy of cardiovascular diseases.

IV. Experimental Protocols

a) Chemistry

The melting point of the compounds was determined in open glass capillary with the help of themionic melting point apparatus and is uncorrected. Elemental analysis of all the newly synthesized compounds were determined by a Perkin-Elimer 2400 elemental analyzer, and results were found within the +/-0.4% of theoretical values. IR spectra were recorded in KBR on a Perkin-Elmer spectrum RX-I, spectrometer. 1H NMR spectra were recorded by Bruker AC-300 F instrument using $CDCl_3$/DMSO-Cl_6 as solvent and tetra methyl silane (TMS) as internal reference standard. All chemical shift values were recorded as δ (ppm). Mass spectra were determined on a VG-70-S instrument.

b) General procedure for the preparation of compounds

i. 6-bromoanthranilic acid (a)

This was prepared according to the method of Wheeler and Oats [14]. Bromine (0.8 mol) in acetic acid (20 ml) was added drop wise to the solution of anthranilic acid (0.4 mol) in absolute AcOH (50 ml). The solid was separated to give 6- bromoanthranilic acid. The solid product thus crystallized out, was washed with water and dried. It was recrystallized from ethanol/water M.P. 208°C, Yield 50%.

ii. Acetanthranils (b)

These were prepared according to the method of Bogert and Soil [15]. A mixture of appropriate anthranilic acid (0.01mol) and acetic anhydride (0.02 mol) were refluxed for 2-3 hours with occasional stirring. The excess of acetic anhydride was distilled off. On cooling, a solid separated out, which was filtered, washed with petroleum ether (40-60 °C) and dried in vacuo. The acetanthranils thus synthesized are given below:

a) Acetanthranils M.P. 78°C
b) 6-Bromoacetanthranils M.P. 172°C

iii. 3-amino-2-methyl monosubstitutedquinazolin-4(3H)-ones: (c)

These were prepared according to the method of Kumar et al [16]. A mixture of appropriate acetanthranils (0.01 mol) and hydrazine hydrate (99%, 0.02 mol) in methanol (dry, 50ml) were refluxed for 8 hours. The excess of solvent was distilled off in vacuo. The residue on cooling gave a crystalline solid, which was recrystallized from methanol-water (1:2).

iv. 3-(ethylacetylamino)-2-methylmono substituted quinazolin- 4 (3H)- one: (1-2)

A mixture of 3-amino-2-methyl mono substitutedquinazolin-4(3H)-one (0.01 mol), ethylchloroacetate (0.01 mol) and anhydrous K_2CO_3 (5.0g) in acetone (dry 80ml) were refluxed for 20 hours on water bath. The

acetone was distilled off and the resulting solid mass poured into water, filtered and the separated solid recrystallized from methanol/water to give compounds (1-2).

v. 3-(hydrazinoacetylamino) - 2 - methylmono substituted-quinazolin – 4 (3H) - one : (3-4)

A mixture of compounds (1-2) (0.01 mol) and hydrazine hydrate (0.02 mol) in methanol (dry, 50ml) were refluxed for 6-8 hours. The excess of solvent was distilled off. On cooling a crystalline solid is obtained which was recrystallized from methanol/water.

vi. 3-(substitutedarylidene)-hydrazinoacetyl-amino-2-methylmono substitutedquinazolin-4(3H)-ones: (5-14)

To a solution of compounds (3-4) (0.01 mol) in absolute ethanol (50 ml), substitutedbenzaldehyde (0.01 mol) and a few drops of glacial acetic acid were refluxed for 8 hours. The solvent was distilled off and the viscous mass thus obtained was
recrystallized from ethanol/water to give compounds (5-14).

vii. 3-[(acetylamino-2-methylmonosubstitutedquinazolin-4(3H)-onyl)]-1'-(substitutedphenyl)-3'-
(substitutedaryl)-formazans: (15-24)

Substituted phenyl (0.01 mol) was dissolved in 4ml glacial acetic acid and 3ml of concentrated HCl was added to 0-5°C. A solution of $NaNO_2$ (1 gm in 5 ml of water) was added drop wise. The diazonium salt solution thus prepared was added with stirring to compounds (5-14) (0.01mol) in 50 ml toluene. During the addition the temperature was maintained below 10°C. The reaction mixture thus obtained was left at room temperature for several hrs and then poured into 250 ml of cold water, The dark red solid which separated out was washed with water, filtered and recrystallized from methanol/water to give compounds (15-24).

viii. 2'-(substitutedaryl)-4'-[3-(acetyl amino-2-methy-lmono substituted quinazolin-4(3H)-onyl)] -5'-(substitutedphenyl)-tetrazolinum chlorides: (25-34)

Compounds (15-24) (0.1 mol) was suspended in ethanol (50 ml) and H_2SO_4 (2N, 5ml) containing a trace of ferrous sulphate and hydrogen peroxide (20%, 10ml). The reaction mixture was refluxed at 100°C for 3 hrs. The tetrazolinum chloride was precipitated by the addition of KCl which was washed with petroleum ether (40-60 °C) and recrystallized from methanol/water to give compounds (25-34).

c) Spectral data of the representative compounds

i. 3-(ethylacetylamino)-2-methyl-6- bromoquinazolin-4(3H)-one: 1

IR (KBr; cm^{-1}): 3250 (NH), 2840 (CH_2), 1730 (C=O), 1550 (C....C of aromatic ring); ^{1}H-NMR ($CDCl_3$): δ9.60 (ss, 1H, N<u>H</u>CH_2), 7.90-7.25 (m, 3H, Ar-H), 4.38(d, 2H, NHC<u>H</u>$_2$), 4.10(q, 2H, J=7Hz, COOC<u>H</u>$_2$CH$_3$), 2.30(s, 3H, CH3), 1.20 (t, 3H, J=7Hz, COOCH$_2$C<u>H</u>$_3$) ppm. MS: $[M]^+$ m/z 340.

ii. 3-(hydrazinoacetylamino)-2-methyl-6-bromo-quinazolin-4(3H)-one: 3

IR (KBr; cm^{-1}): 3340 (N<u>H</u>NH_2), 3040 (aromatic C-H), 2850 (CH_2), 1720 (C=O), 1560 (C....C of aromatic ring), 600 (C-Br stretch); ^{1}H-NMR ($CDCl_3$): δ9.55 (ss, 1H, N<u>H</u>CH_2), 7.80-7.20 (m, 3H, Ar-<u>H</u>), 5.25 (brs, 1H, N<u>H</u>NH_2), 4.30 (d,2H, NHC<u>H</u>$_2$), 2.50 (ss, 2H, N<u>H</u>$_2$), 2.35 (s, 3H, C<u>H</u>$_3$) ppm. MS: $[M]^+$ m/z 326.

iii. 3-(p-hydroxybenzylidene)-hydrazinoacetylamino-2-methyl-6-bromo-quinazolin-4(3H)-one: 9

IR (KBr; cm^{-1}): 3240 (NH), 3030 (aromatic C-H), 1700 (C=O), 1630 (C=N), 1560 (C....C of aromatic ring); ^{1}H-NMR ($CDCl_3$): δ9.45 (ss, 1H, N<u>H</u>CH_2), 9.0 (s, 1H, O<u>H</u>), 8.85 (s, 1H, N=C<u>H</u>-Ar), 8.00-7.10 (m, 7H, Ar-<u>H</u>), 6.40 (ss, 1H, CON<u>H</u>), 4.40 (d, 2H, NHC<u>H</u>$_2$), 2.30 (s, 3H, C<u>H</u>$_3$) ppm. MS: $[M]^+$ m/z 430.

iv. 3-[(acetylamino-2-methyl-6-bromo-quinazolin-4(3H)-onyl)]-1'-(o-chloroaniline)-3'-(p-hydroxyphenyl)-formazan: 19

IR (KBr; cm^{-1}): 3248 (NH), 3040 (aromatic C-H), 1720 (C=O), 1640 (C=N), 1550 (C....C of aromatic ring), 1425 (N=N), 1260 (C-N); ^{1}H-NMR ($CDCl_3$): δ9.50 (ss, 1H, N<u>H</u>CH_2), 9.04 (s, 1H, OH), 8.20-7.00 (m, 11H, Ar-<u>H</u>), 6.45 (ss, 1H, CON<u>H</u>), 4.40 (d, 2H, NHC<u>H</u>$_2$), 2.25 (s, 3H, C<u>H</u>$_3$) ppm. MS: $[M]^+$ m/z 568.

v. 2'-(p-hydroxyphenyl)-4'-[3-acetylamino-2-methyl-6-bromo-quinazolin-4(3H)-onyl)]-5'-(o-chloroaniline)-tetrazolinum chloride: 29

IR (KBr; cm^{-1}): 3245 (NH), 3053 (aromatic C-H), 2853 (CH_2), 1740 (C=O), 1640 (C=N), 1580 (C....C of aromatic ring), 1520 (N-N), 1420 (N=N), 1250 (C-N); ^{1}H-NMR ($CDCl_3$): δ9.30 (ss, 1H, N<u>H</u>CH_2), 9.0 (s, 1H, OH), 8.30-7.50 (m, 11H, Ar-<u>H</u>), 4.35 (d, 2H, NHC<u>H</u>$_2$), 2.30 (s, 3H, C<u>H</u>$_3$) ppm. MS: $[M]^+$ m/z 603.

vi. Cardiovascular activity

Preliminary cardiovascular activity tests were carried out on albino rats 100-120g of either sex (the pregnancy was excluded) for all the synthesized indole derivatives. The newly synthesized compounds (test drugs) were administered intravenously (from right femoral vein) by dissolving them in propylene glycol and the effect on blood pressure (B.P), heart rate (HR) and pressor responses evoked either by carotid occlusion (CO) or intravenous noradrenalin (NA) 1-2 µg/Kg injection was observed. Injection of .20 mL of propylene glycol induced a mild and transient decrease of 1-2 mmHg in blood pressure without affecting the CO and NA response. The blood pressure was recorded from the left common carotid artery by means of a mercury manometer from femoral artery on one channel of "Encardiorite" (India) polygraph using stathus P25

transducer. Electrocardiogram (Lead II) was recorded on one channel of "Encardiorite" (India) polygraph in all the experiments.

vii. Acute toxicity study

The toxicity study was carried out on Charles foster mice of either sex (pregnancy was excluded). Approximate 50% lethal dose (ALD50) of the promising compounds was determined in albino mice. The mice of either sex weighing between 18-25 g were used for the study. The drugs were injected by intraperitonial (i.p.) route at different dose levels in separate groups of animals. After 24 hours of drugs administration, percent mortality in each group was observed. From the data obtained, ALD50 was calculated by using Smith method [17].

V. Acknowledgement

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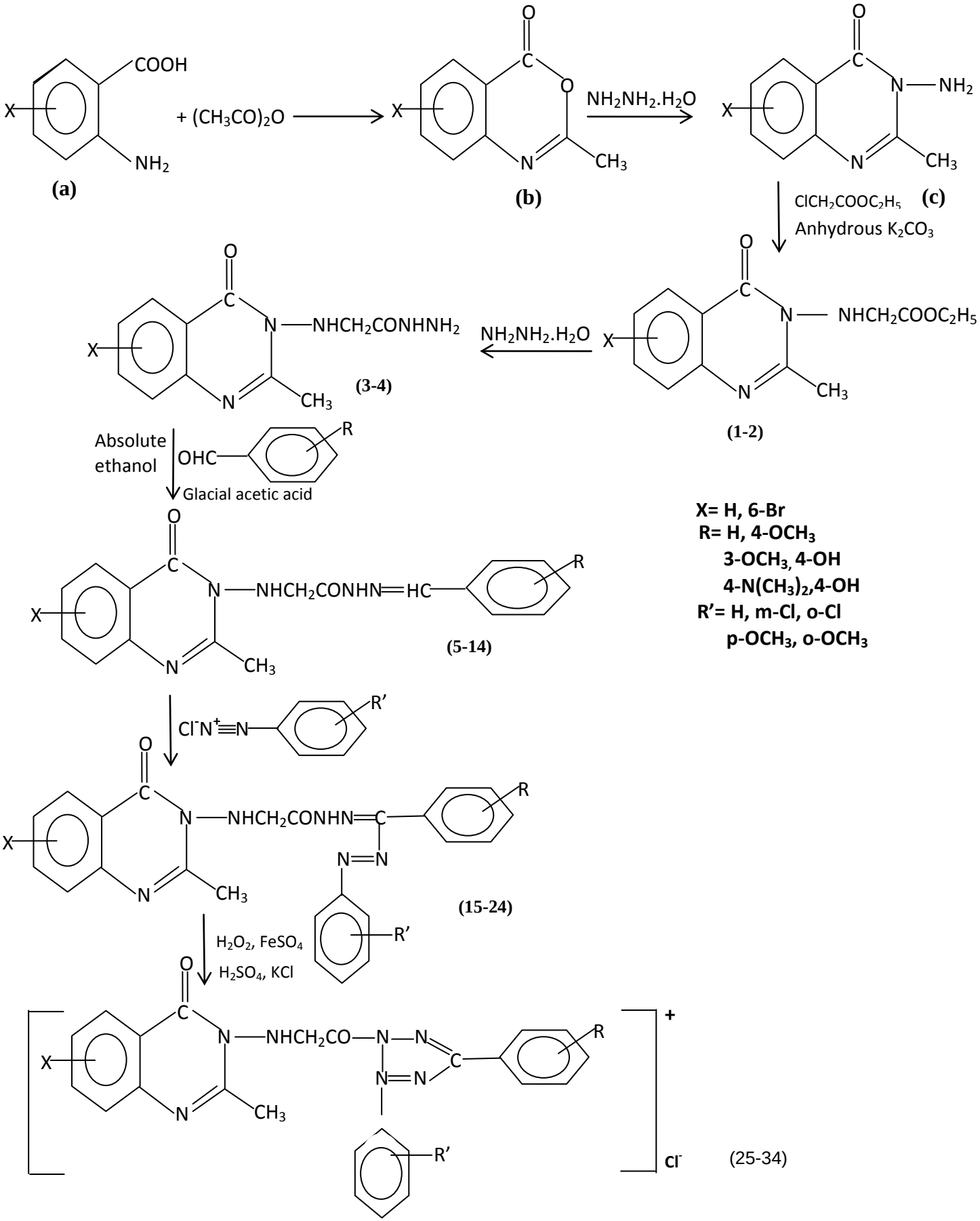
SCHEME I



Scheme I. **Synthetic pathway for the preparation of quinazolinone derivatives**

Table 1 : Yield and elemental analysis of compounds

Co-mpd.	X	R	R'	M.P. (ºC)	Yield (%)	Recrystal llisation Solvent	Molecular Formula	Element Analysis (%) Calcd.(Found) C	H	N
1	6-Br	-	-	160	65	Methanol/Water	$C_{13}H_{14}N_3O_3Br$	45.88(45.90)	4.11(4.14)	12.35(12.37)
2	H	-	-	120	60	Methanol/Water	$C_{13}H_{15}N_3O_3$	59.77(59.72)	5.74(5.70)	16.09(16.06)
	6-Br	-	-	220	60	Methanol/Water	$C_{11}H_{12}N_5O_2Br$	40.49(40.45)	3.68(3.65)	21.47(21.45)
3	H	-	-	180	55	Methanol/Water	$C_{11}H_{13}N_5O_2$	53.44(53.40)	5.26(5.30)	28.34(28.30)
	6-Br	H	-	170	42	Methanol/Water	$C_{18}H_{16}N_5O_2Br$	52.17(52.14)	3.86(3.83)	16.90(16.92)
5	6-Br	$4\text{-}OCH_3$	-	182	45	Acetic acid/Water	$C_{19}H_{18}N_5O_3Br$	49.56(49.52)	3.91(3.88)	15.21(15.23)
7	6-Br	$3\text{-}OCH_3$, 4-OH	-	240	40	Benzene/Hexane	$C_{19}H_{18}N_5O_4Br$	49.56(49.52)	3.91(3.88)	15.21(15.23)
8	6-Br	$4\text{-}N(CH_3)_2$	-	186	42	THF	$C_{20}H_{21}N_6O_3Br$	52.51(52.54)	4.59(4.63)	18.38(18.35)
9	6-Br	4-OH	-	198	45	Ethanol/Water	$C_{18}H_{16}N_5O_3Br$	50.23(50.20)	3.72(3.70)	16.27(16.30)
10	H	H	-	170	48	Petroleum/Ether	$C_{18}H_{17}N_5O_2$	64.47(64.45)	5.07(5.04)	20.89(20.92)
11	H	$4\text{-}OCH_3$	-	194	46	Ethanol	$C_{19}H_{19}N_5O_3$	62.46(62.44)	5.20(5.24)	19.17(19.20)
12	H	$3\text{-}OCH_3$, 4-OH	-	200	42	Benzene	$C_{19}H_{19}N_5O_4$	59.84(59.80)	4.98(4.94)	18.37(18.40)
13	H	$4\text{-}N(CH_3)_2$	-	160	45	Benzene	$C_{20}H_{22}N_6O_2$	63.49(63.52)	5.82(5.80)	22.22(22.25)
14	H	4-OH	-	170	45	Acetone	$C_{18}H_{17}N_5O_3$	61.53(61.57)	4.84(4.82)	19.94(19.90)
15	6-Br	H	m-Cl	142	40	Methanol/Water	$C_{24}H_{19}N_7O_3ClBr$	52.12(52.10)	3.43(3.46)	17.73(17.70)
16	6-Br	$4\text{-}OCH_3$	H	195	42	Methanol/Water	$C_{25}H_{22}N_7O_4Br$	54.74(54.78)	4.01(4.05)	17.88(17.04)
17	6-Br	$3\text{-}OCH_3$, 4-OH	o-Cl	238	40	Methanol/Water	$C_{25}H_{21}N_7O_4BrCl$	50.12(50.16)	3.50(3.54)	16.37(16.35)
18	6-Br	$4\text{-}N(CH_3)_2$	$p\text{-}OCH_3$	210	40	Methanol/Water	$C_{27}H_{27}N_8O_3Br$	54.82(54.80)	4.56(4.52)	18.95(18.98)
	6-Br	4-OH	o-Cl	230	40	Methanol/Water	$C_{24}H_{19}N_7O_3ClBr$	50.65(50.62)	3.34(3.37)	17.23(17.23)
19	H	H	$o\text{-}OCH_3$	190	42	Methanol/Water	$C_{25}H_{23}N_7O_3$	63.96(63.98)	4.90(4.94)	20.89(20.86)
21	H	$4\text{-}OCH_3$	o-Cl	185	45	Methanol/Water	$C_{25}H_{22}N_7O_3Cl$	59.58(59.56)	4.36(4.34)	19.46(19.48)
22	H	$3\text{-}OCH_3$, 4-OH	H	235	40	Methanol/Water	$C_{25}H_{23}N_7O_4$	61.85(61.88)	4.74(4.72)	20.20(20.22)
23	H	$4\text{-}N(CH_3)_2$	m-Cl	142	40	Methanol/Water	$C_{26}H_{25}N_8O_2Cl$	60.40(60.42)	4.84(4.88)	21.68(21.70)
24	H	4-OH	$o\text{-}OCH_3$	195	45	Methanol/Water	$C_{25}H_{23}N_7O_4$	61.85(61.88)	4.74(4.78)	20.20(20.24)
25	6-Br	H	m-Cl	165	30	Methanol/Water	$C_{24}H_{18}N_7O_2Cl_2Br$	49.06(49.08)	3.06(3.02)	16.69(16.65)
26	6-Br	$4\text{-}OCH_3$	H	220	30	Methanol/Water	$C_{25}H_{21}N_7O_3ClBr$	51.50(51.54)	3.60(3.58)	16.82(16.84)
27	6-Br	$3\text{-}OCH_3$, 4-OH	o-Cl	250	35	Methanol/Water	$C_{25}H_{21}N_7O_4Cl_2Br$	47.31(47.35)	3.31(3.35)	15.45(15.42)
28	6-Br	$4\text{-}N(CH_3)_2$	$p\text{-}OCH_3$	270	38	Methanol/Water	$C_{27}H_{26}N_8O_3ClBr$	51.79(51.75)	4.15(4.12)	17.90(17.94)
	6-Br	4-OH	o-Cl	240	38	Methanol/Water	$C_{24}H_{18}N_7O_3Cl_2Br$	47.76(47.72)	2.98(2.95)	16.25(16.23)
29	H	H	$p\text{-}OCH_3$	200	38	Methanol/Water	$C_{25}H_{23}N_7O_3Cl$	59.98(59.55)	4.36(4.38)	19.46(19.42)
31	H	$4\text{-}OCH_3$	o-Cl	225	38	Methanol/Water	$C_{25}H_{21}N_7O_3Cl_2$	55.76(55.72)	3.90(3.94)	18.21(18.25)
32	H	$3\text{-}OCH_3$, 4-OH	H	244	35	Methanol/Water	$C_{25}H_{22}N_7O_4Cl$	57.74(57.78)	4.23(4.20)	18.86(18.82)
33	H	$4\text{-}N(CH_3)_2$	m-Cl	165	30	Methanol/Water	$C_{26}H_{24}N_8O_2Cl_2$	56.62(56.65)	4.35(4.32)	20.32(20.30)
34	H	4-OH	$o\text{-}OCH_3$	240	35	Methanol/Water	$C_{25}H_{22}N_7O_4Cl$	57.74(57.70)	4.23(4.27)	18.86(18.84)

Table 2 : Cardiovascular activity of the synthesized compounds

Co-mpd.	X	R	R'	Dose Mg/kg i.v.	Change in mean blood pressure mmHg Control Mean± SE	Immediate Mean± SE	Delayed Mean± SE	Duration in minutes Mean± SE	Change in resting HR bpm	Effect on pressure responses CO	NA	ALD_{50} mg/kg p.o.
5	6-Br	H	-	2.5	138±11.51	118.6±12.42*	127±12.02	49.6±1.67	-	Inhibited	Inhibited	>1000
6	6-Br	$4\text{-}OCH_3$	-	1.25	151±5.94	136±4.18***	106±4.04***	72.4±6.38	-	Inhibited	Inhibited	
				2.5	154.4±4.98	119±5.76***	83.8±4.52***	95±5.00	-	Inhibited	Inhibited	>2000
				5.0	158.4±5.87	108.4±6.68***	58.4±5.20***	134.8±7.59	-	Inhibited	Inhibited	
7	6-Br	$3\text{-}OCH_3$, 4-OH	-	2.5	138.4±8.96	114±7.48**	98.4±9.00***	45.2±2.16	-	Inhibited	Inhibited	>1000
8	6-Br	$4\text{-}N(CH_3)_2$	-	2.5	138.2±11.54	104.6±11.61**	106.2±12.77***	60.8±1.09	Potentiated 1-2 bpm	Inhibited	-	>1000
9	6-Br	4-OH	-	2.5	146±7.41	106.8±6.18**	126.2±5.93**	56.67±2.81	Potentiated 3 bpm	Inhibited	-	>1000
10	H	H	-	2.5	145.5±7.46	116.6±5.77***	96.6±5.60***	74.4±3.08	-	Inhibited	-	>1000
11	H	$4\text{-}OCH_3$	-	2.5	145.6±6.50	126.2±4.32	105±4.04***	59.8±2.86	-	Blocked	-	>1000
12	H	$3\text{-}OCH_3$, 4-OH	-	2.5	141±13.87	91.6±13.84***	118.4±16.19	59±2.64	-	Inhibited	-	>1000
13	H	$4\text{-}N(CH_3)_2$	-	1.25	159.6±7.30	124.6±8.73***	89.6±9.81***	99.6±2.96	-	Inhibited	-	
				2.5	156.4±6.98	104.2±7.38***	55.8±6.79***	128.2±2.86	-	Inhibited	-	>2000
				5.0	169.2±7.80	90.8±9.09	38.8±7.67***	195±4.12	-	Inhibited	-	
14	H	4-OH	-	2.5	143.2±8.16	93.2±5.54***	133.2±6.45***	60.2±1.67	-	Blocked	-	>1000
15	6-Br	H	m-Cl	2.5	136±4.18	-	76±3.80***	30.6±1.94	-	–	–	>1000
16	6-Br	$4\text{-}OCH_3$	H	2.5	134±4.18	-	69±7.21***	29.2±2.28	-	–	–	>2000
17	6-Br	$3\text{-}OCH_3$, 4-OH	o-Cl	2.5	139±9.61	-	99.8±9.98***	42.33±2.51	-	-	-	>1000
18	6-Br	$4\text{-}N(CH_3)_2$	$p\text{-}OCH_3$	2.5	138.2±11.54	-	104.6±11.61**	29±2.64	-	-	-	>1000
19	6-Br	4-OH	m-Cl	2.5	137.4±6.06	-	120±7.21***	23.8±2.77	-	-	-	>1000
20	H	H	$p\text{-}OCH_3$	2.5	139±9.61	-	99.8±9.98***	22.6±3.97	-	-	-	>1000
21	H	$4\text{-}OCH_3$	o-Cl	2.5	134.2±13.04	-	105±11.57***	39.2±2.20	-	-	-	>1000
22	H	$3\text{-}OCH_3$, 4-OH	H	2.5	137.6±11.67	-	95±12.18***	21.6±2.70	-	-	-	>1000
23	H	$4\text{-}N(CH_3)_2$	m-Cl	2.5	138.4±8.97	-	104.6±9.01***	29.6±1.67	-	-	-	>1000
24	H	4-OH	$o\text{-}CH_3$	2.5	135±9.61	-	93.4±9.86***	30±1.41	-	-	-	>1000
25	6-Br	H	m-Cl	2.5	132±8.13	-	122±8.68	9.6±1.81	-	–	–	>1000
26	6-Br	$4\text{-}OCH_3$	H	2.5	133.8±8.37	-	116.4±6.50**	14±3.08	-	–	–	>2000
27	6-Br	$3\text{-}OCH_3$, 4-OH	o-Cl	2.5	132.2±10.77	-	120.2±13.40*	9.6±2.60	-	-	-	>1000
28	6-Br	$4\text{-}N(CH_3)_2$	$p\text{-}OCH_3$	2.5	132±9.61	-	115.2±10.18*	12±1.87	-	-	-	>1000
29	6-Br	4-OH	m-Cl	2.5	132.2±10.77	-	117±9.54*	14±3.08	-	-	-	>1000
30	H	H	$p\text{-}OCH_3$	2.5	130.6±7.53	-	120.4±6.73*	13.6±2.70	-	-	-	>1000
31	H	$4\text{-}OCH_3$	o-Cl	2.5	132.2±6.01	-	127.2±6.37*	5.2±1.48	-	-	-	>1000
32	H	$3\text{-}OCH_3$, 4-OH	H	2.5	132±9.46	-	123±8.68***	5.8±1.92	-	-	-	>1000
33	H	$4\text{-}N(CH_3)_2$	m-Cl	2.5	133.6±6.10	-	128.6±5.77*	8.8±3.11	-	-	-	>1000
34	H	4-OH	$o\text{-}CH_3$	2.5	133.8±9.90	-	121.4±9.60*	10±1.58	-	-	-	>1000

* $p > 0.05$; ** $p < 0.01$; *** $p < 0.001$

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Neurotoxic Syndromes Sequencially Occuring after Consumption of organophosphorus Compound - A Case Report

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Abstract- Organo-phosphorus compounds (OPC) are among the most used poisons for suicide in India, and associated toxic syndromes are well described. We report a young man who presented to us with alleged consumption of chlorpyrifos, a crystalline organophosphate insecticide. During hospitalization he developed acute organo-phosphorus toxicity (type I) and intermediate syndrome (type II), both situations were managed by assisted ventilation and supportive care. After 6 weeks of discharge he reported with features of delayed poly neuropathy (type III). It is rare for these patients to follow up at the same centre to identify and manage these toxicities. Often the type III toxicity is misdiagnosed and over investigated for other causes of neuropathy. Though OPC poisoning is commonly encountered in practice, only few reports have described all toxicities to occur in the same patient.

GJMR-B Classification : *NLMC Code: WL 141, WL 140*

Neurotoxic Syndromes Sequencially Occuring after Consumption of organophosphorus Compound - A Case Report

Peter George [α] & NarasimhaHegde [σ]

Abstract- Organo-phosphorus compounds (OPC) are among the most used poisons for suicide in India, and associated toxic syndromes are well described. We report a young man who presented to us with alleged consumption of chlorpyrifos, a crystalline organophosphate insecticide. During hospitalization he developed acute organo-phosphorus toxicity (type I) and intermediate syndrome (type II), both situations were managed by assisted ventilation and supportive care. After 6 weeks of discharge he reported with features of delayed poly neuropathy (type III). It is rare for these patients to follow up at the same centre to identify and manage these toxicities. Often the type III toxicity is misdiagnosed and over investigated for other causes of neuropathy. Though OPC poisoning is commonly encountered in practice, only few reports have described all toxicities to occur in the same patient.

I. Introduction

According to the report of Accidental deaths and suicides in India, 2009, Government of India, 127151 people committed suicide in 2009[1]. OPC poisoning is the most commonand account to half of hospital admissions due to poisoning in India. Its ready availability and easy accessibilitypossibly makes it the most used suicidal agents in India[2], [3] ,[4], [5]. The neurological toxicities of OPC poisoning can be Type I syndrome or cholinergic crisis, Type II syndrome or Intermediate syndromeand Type III syndrome or organophosphate induced delayed neuropathy (OPIDN) [6], [7], [8].A delayed organo-phosphorus compounds induced neuropsychiatric syndrome due to chronic poisoning has also been described in literature. In this report we identified all three described types of neurotoxicsyndromes due to OPC in the same individual.

II. Case Report

We report an interesting case of OPC poisoning who developed all the described toxic syndromes. A 36 year old man was admitted to our hospital with alleged consumption of Chlorpyrifos(250 ml), an organo-phosphorus compound, with suicidal intention. He had features of type I toxicity at admission, and managed after decontamination, with atropine, prali-doxime and supportive care. On day - 6 of admission he developed neckand respiratory muscle weakness, followed by weakness in allhis limbs. This being typical of Type - 2 syndrome (intermediate syndrome), was managed with assisted ventilation and supportive care. On day - 16 he was weaned off from assisted ventilation and on day -21 was discharged from hospital with no obvious neurological deficits.

He was readmitted to hospital after 4 weeks of discharge with history of progressive difficulty in griping objects with hand, walking and getting up from squatting position. He had distal paraesthesia in all limbs, but had no sphincter disturbances. On examination, vital signs and cranial nerves were normal, with no disturbances in autonomic or higher mental functions. There was wasting of distal muscles in all limbs; and had weak hand grip and plantar movements. Clinically, he had distal hypotonia and proximal hypertonia in the limbs. His deep tendon jerks were exaggerated, except for ankle jerks which were absent; and all modalities of sensations were normal. The routine haematology and biochemistry, including electrolytes, CPK and thyroid functions, were normal. Nerve conduction studies revealed predominantly axonal type of sensory- motor polyradiculoneuropathy in both lower limbs. He was treated with a short course of glucocorticoids, high doses of neurotropic vitamins, and physical therapy. He had little improvement even after weeks of treatment.

III. Discussion

OPC poisoning cause inhibition of acetylcholinesterase (AChE), leading to the accumulation of acetylcholine (ACh) in the body. OPC's are being used for over 70 years and are the most used insecticides world-over, including India [2], [4], [5]. The indiscriminate use of these compounds over the decades has resulted in innumerable toxicities to humans as well animals. Suicidal and occupational OP poisoning in agricultural workers was prevalent in developing countries, whereas accidental OP poisoning was prevalent in developed countries [7].

The Accidental deaths and suicides in India, 2009, Ministry of Home Affairs, Government of India,

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poisoning with pesticides contributed to 20.4 % of suicides in India, with most arising out of family problems[1]. OPC's are the most commonly used suicidal agent in India[2], [4], [5].OPC poisoning occurs from gastrointestinal tract following suicidal consumption or absorption through skin, mucous membranes and respiratory tract following accidental exposure[5].

These compounds after absorption are hydrolysed by esterases, and competitively bind to the esteratic site of the enzyme acetylcholinesterase (AChE), resulting in its phosphorylation. Depending on the compound involved the binding may be stable and takes hours or weeks disintegrate. The accumulation of excess acetylcholine (ACh) at the cholinergic nerve endings result in the characteristic clinical manifestations[2], [3], [6].

The toxic syndromes after acute exposure to OPC are well described in literature. Type I and II toxicities, the cholinergic and intermediate syndrome respectively are common in the emergency department. After acute exposure of OPC, type III toxicity or organophosphate-induced delayed polyneuropathy (OPIDP) is also described. A chronic organophosphate-induced neuropsychiatric disorder (COPIND), at times described as type IV toxicity, occurs in chronic OPC exposure.

Type I or cholinergic syndrome results from excessive stimulation of muscarinic receptors, resulting in bradycardia, diarrhoea, vomiting, fasciculation, sweating, salivation and micturition[6], [7], [8]. Type 2 or Intermediate syndrome follow type I toxicity, and occurs due to excessive Ach at the neuromuscular junction causing down-regulation of nicotinic receptors resulting dysfunction of neuromuscular junction. Develop in about 20%-50% of cases depending on the ingested quantity, its duration, and the compound. Usually occur 24 to 96 hours after the recovery from the cholinergic crisis. It is marked by predominant proximal limb muscles and neck flexor weakness, with or without cranial-nerve palsies. The intermediate syndrome may last from 5 to 18 days[9], [10].

As with other poisoning the first step in the management of these patients is gastrointestinal and skin decontamination. Atropine is used as antidote, in type I syndrome, to counter the muscarinic effects of acetylcholine. Pupillary dilation, drying up of secretions, tachycardia and fever are features of atropinisation. Once achieved, should be maintained for 3-5 days, depending upon the clinical situation. If respiratory muscule paralysis supervenes, mechanical ventilation must be instituted. Type II syndrome is supportive with mechanical ventilation and recovery is the rule, muscles of respiration being the last to recover[11], [12]. The use of oximes in cholinergic phase as rejuvenators of the enzyme cholinesterase is controversial [13].

Our patient had features of type - I toxicity at admission, and after decontamination was managed with atropine, pralidoxime and supportive care. He did not require assisted ventilation during the type I toxicity. On day - 6 of admission he developed neck and respiratory muscle weakness, followed by weakness in all his limbs; typical of Type - 2 syndrome. He required assisted ventilation for 10 days along with supportive care. He responded well, and on day -21 was discharged from hospital with no neurological deficits. As described, our patient also developed the type I & II syndromes and recovered well with the supportive management.

Type III syndromes, often named as organophosphate induced delayed neuropathy (OPIDN), usually occurs about 1-3 weeks after consumption of OPC. It occurs from phosphorylation and inhibition of neuropathy target esterase (NTE) in axons causing degeneration of long axons. This is often a pure motor or axonal neuropathy[9].

Our patient reported to hospital after 4 weeks with difficulty in griping objects with hand; walking and getting up from squatting position; distal paraesthesia of the limbs, but had no sphincter disturbances. He was found to have a mixture of upper and lower motor neuron signs on examination, which was classical of type III toxicity. There are a few reports of type III syndromes in literature resulting from chlorpyrifos and other organo-phosphorus compounds[14][15][16]. In addition to these neurological syndromes following acute exposure; individuals with low dose chronic exposure, develop several neuro-behavioural changes termed together as 'chronic organophosphate induced neuropsychiatric disorders' (COPIND) or type IV syndrome.

The researches with newer molecules and drugs have been inspiring in last decades. Bioscavengers, nano carriers, recombinant bacterial phosphodiesterases have been very encouraging in recent years. Alkalanisation, intravenous magnesium sulphate, hemofiltration and antoxidants have shown to reduce neurotoxicity. The recent advances in treatment of organophosphorouspoisoning must be accessible to clinicians for its morbidity, magnitude and socio-economic implications [3], [17].

Even though we encounter many organo-phosphorus compound poisoning in practice with type I & II toxicities, type III is very rare. The chances of overlooking type III syndrome are high, as it is uncommon and also due to similarity with other neuropathies. We were able to identify type III syndrome as the patient reported back for follow up. He was managed with a short course of glucocorticoids, high dose neurotropic vitamins, and physical therapy; but had little improvement after weeks of treatment[11], [12].

IV. Conclusion

Organo-phosphorus compound consumption is among the commonest poisoning, and the treatment outcome for type I & II syndromes are excellent with early identification and institution of treatment. The regular follow up of patients after discharge from hospital could possibly identify the type III and IV syndromes. No specific treatment exists to prevent occurrence of the neuropathy following exposure.

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Natural Antioxidants and their Intrinsic Worth for Wellbeing

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Abstract- Free radicals produced on exposure to sunlight, X-rays, ozone, tobacco smoke, automobile exhaust, environmental pollutants, and by several other physiological processes, are highly reactive and can damage nucleic acids, proteins, lipids and carbohydrates that subsequently affect the immune functioning causing degenerative diseases. In a normal cell there is an appropriate balance between pro-oxidant and antioxidants. When the level of pro-oxidants is increased in comparison to antioxidant, this state is termed as oxidative stress (OS). It is imposed on the cells due to increase in oxidant generation and decrease in antioxidants protection, resulting in failure in repair of oxidative damage. Exposure to pathogens, inappropriate lifestyle, excessive exercise, and by-products of normal metabolism are also contributing factors to OS. Reactive oxygen species are also responsible for DNA (deoxyribose nucleic acid) damages resulting in mutagenic changes that are responsible for several diseases. Oxidative stress deregulates the cellular functions leading to neuro-degenerative diseases, gastroduodenal pathogenesis, some kinds of cancer, cataracts, premature aging, inflammation, cardiovascular, and metabolic dysfunction.

Keywords: *phytochemicals; allicin; genestein; glucomannans; RDA; pdcaasw; peroxidation; ROS: free radicals; nutritive value; epidemiological data.*

GJMR-B Classification : *NLMC Code: QV 325*

Strictly as per the compliance and regulations of:

Natural Antioxidants and their Intrinsic Worth for Wellbeing

Bina Rani [α], Upma Singh [σ] & Raaz K Maheshwari [ρ]

Abstract- Free radicals produced on exposure to sunlight, X-rays, ozone, tobacco smoke, automobile exhaust, environmental pollutants, and by several other physiological processes, are highly reactive and can damage nucleic acids, proteins, lipids and carbohydrates that subsequently affect the immune functioning causing degenerative diseases. In a normal cell there is an appropriate balance between pro-oxidant and antioxidants. When the level of pro-oxidants is increased in comparison to antioxidant, this state is termed as oxidative stress (OS). It is imposed on the cells due to increase in oxidant generation and decrease in antioxidants protection, resulting in failure in repair of oxidative damage. Exposure to pathogens, inappropriate lifestyle, excessive exercise, and by-products of normal metabolism are also contributing factors to OS. Reactive oxygen species are also responsible for DNA (deoxyribose nucleic acid) damages resulting in mutagenic changes that are responsible for several diseases. Oxidative stress deregulates the cellular functions leading to neuro-degenerative diseases, gastroduodenal pathogenesis, some kinds of cancer, cataracts, premature aging, inflammation, cardiovascular, and metabolic dysfunction. It may also influence the immune system either by hyper-excision to cause autoimmune disorder, or suppress it, resulting in the high susceptibility to infection. Owing to increased safety concerns about synthetic antioxidants, exploitation of safer antioxidants based on natural origin is the focus of research nowadays, and the present study has hallmarked the same. In this manuscript, therapeutic worth of various constituents including antioxidants of natural products viz. garlic, soyabean, gooseberry, broccoli, spirulina and aloevera have been delineated precisely.

Keywords: phytochemicals; allicin; genestein; glucomannans; RDA; pdcaasw; peroxidation; ROS: free radicals; nutritive value; epidemiological data.

I. Introduction

Antioxidants are vitamins or nutrients that may help to prevent the damaging effects of oxidation on your body's organs and tissues [1].

They achieve this by protecting the cells of human body from the damage done by "free radicals". Protection of body cells is the main feature and root cause for all benefits of antioxidants. What are free radicals, and how do they damage cells? From the name "Anti-oxidant" you can see that the function of these nutrients is somehow in their opposition to oxidation. You might guess that the benefits of antioxidants are somehow related to "fighting" oxidation as if it were a bad thing...

Well, here is the situation. The oxidation process can be described as the "flame of life". Oxidation is the change in a chemical when its atoms lose their electrons. This process constantly occurs in order to produce energy within our body. The natural by-products of this process are free radicals: atoms which lack electrons. These free radicals cause aging and other complications. The older we become, the larger the amount of free radicals which may be accumulated in our bodies - and the possibility of resultant cell damage becomes more severe. Free radicals are reactive chemical species that differ from other compounds in that they have unpaired electrons in their outer orbitals. They are capable of damaging cellular components, and accumulating evidence suggests they may contribute to various disease entities. Biologic systems are exposed to free radicals that have been formed endogenously or that result from external influences such as ionizing radiation. Oxygen free radicals are continuously being produced intracellularly by oxidation-reduction reactions. The sequential univalent reduction of molecular oxygen initially forms the superoxide anion radical, which in turn is converted, in the presence of transition metal ions, into the highly reactive hydroxyl radical [2-4].

Free radicals are detected by electron spin resonance spectroscopy, but often this procedure is difficult to use for study of free radical involvement in biologic systems, and investigators have resorted to inferring their presence by identifying the products of free radical reactions. All aerobic organisms possess substances that help prevent free radical-mediated injury. These include antioxidants such as vitamin E and the enzymes superoxide dismutase and glutathione peroxidase. Free radicals damage cells and cell membranes because they "steal" electrons from cell molecules. This change may result in a chain reaction, in which more and more molecules will lose their electrons. This makes the whole cell work in a "wrong" way - and this is what may cause a disease! Of course, just one single cell would not make much of difference to anyone's health. Many cells would need to be damaged

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before any symptoms will appear. There are many factors in our environment which contribute to an increase of free radicals in our bodies [5, 49-55].

External damaging free radicals are derived from the elements we live with, such as chlorine in the water we drink, chemicals in the food we eat, smoking, polluted air we breathe and radiation from the sun or other sources like power lines, electromagnetic waves etc. Free radicals can steal an electron and break down another biomolecule such as loose proteins, sugars, fatty acids, etc. that are not part of a larger chemical structure. In these cases the free radical does little damage. If a free radical steals an electron from one of the proteins that is contained in a strand of collagen (rather than a loose protein), it causes a change in the chemical structure of the collagen at that point and causes a break in the collagen strand. This is damage. Once a bundle of collagen has multiple points of damage which occurs over years, the strand of collagen becomes dysfunctional and loses its elastic quality6. The skin begins to sag. Over time free radical damage happens to the various components of the body and this damage is progressive. Free radicals chip away at cell walls, molecule by molecule, making holes. The cells leak and lose their chemical balances. Subsequent free radicals are able to chip away at DNA, making cells dysfunctional. If this damage affects cellular DNA, the cell may malfunction and this is what happens cell by cell over the lifetime of a human being, ultimately causing entire organs to malfunction, because their cells malfunction. If the DNA of basal keratinocytes, for example, are damaged the cells may become dysfunctional and the basal cells will reproduce cells that are equally as damaged and dysfunctional, resulting in the aging and dysfunction of the skin and its various components. Aging is simply the progression of damage, caused by free radicals [7-9, 56-61].

The major creators of free radicals in the skin are (1. normal chemical processes such as producing and using energy, producing skin components such as lipids, and other daily chemical processes that give off free radicals as a natural byproduct (2. unprotected sun exposure, (3. products applied to the skin that produce free radicals and (4. pollution. When acne is involved, acne becomes another creator of free radicals and in the case of moderate to severe acne, assumes the second position, ahead of unprotected sun exposure. Most of the chemical processes that occur in the skin, emit free radicals. In the body, the processing of food, producing energy and using energy creates free radicals. Breathing and using our muscles to perform functions creates free radicals. Manufacturing collagen or lipids or pigment produces free radicals. These free radicals can create damage to the components of the skin as they steal an electron from another component to make themselves complete and stable. When acne infections occur, the skin generates hydrogen peroxide to kill bacteria. Hydrogen peroxide gives peroxide free radical and damages the components of the skin. The infections destroy skin components and all of these components must be repaired or reproduced. This again generates volumes of chemical processes that generate additional volumes of damaging free radicals [10, 62-68].

One way to protect cells from free radicals is to provide our bodies with molecules which can be used as targets for oxidation - diverting their "attention" from the molecules that make cells and membranes! These special molecules are antioxidants: they are able easily to lose, or to accept electrons, with no harm done.

So, the major feature of antioxidants is that they neutralize free radicals, thus preventing potential damage. All the benefits of antioxidants are the result of this feature. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet [11-12]. Antioxidants destroy free radicals"...

In recent years, a new term ‘neutraceuticals’ has been coined, which combines ‘nutrition’ and ‘pharmaceutical’ to mean that they have health-enhancing role or physiologically active food components that can have certain prophylactic and / or healing properties and can be used as preventive drugs or as food supplements, Stephen de Felice, Director of NYFIM (New York’s Foundation for Innovation in Medicine) is credited with the first use of the term neutraceutical. These compounds include disease preventing phytochemicals or phytonutrients present in food stuffs; for example, isoflavones in soyabean, lycopene in tomatoes, lignans in flaxseed, and sulphoraphane in broccoli, which have protective effect against cancer [14-16]. In future, phytochemicals of neutraceutical importance may be used as preventive medicine. Growing evidence indicates that antioxidants can scavenge free radicals and offer protection against a variety of diseases. Antioxidants are known to diffuse the volatile toxic molecules of ROS and protect lung tissue from their toxic effects. Phytochemicals such as carotenoids, limonoids, tocopherols, ascorbates, lipoic acid, and polyphenols are strong natural antioxidants generally found in plants and foods that play an important role in human health [17, 20, 63].

Carotenoids: Terpenes are the largest class of phytochemicals, with carotenoids and limonoids being its two major subclasses. There are more than 700 naturally occurring carotenoids that acts as biological

antioxidants and protect cells and tissues from the damaging effects of free radicals. Carrots, tomatoes, parsley, papaya, orange and green leafy vegetables like amaranth, chenopods, mustard, fenugreek, spinach, cabbage, radish and turnip are rich sources of carotenoids. They have been classified into two major groups on the basis of their structure (i) carotenes (β-carotene, lycopene) containing only carbon and hydrogen that may be cyclic or linear; and (ii) oxycarotenoids (xanthophylls, lutein) containing carbon, hydrogen and oxygen in the form of hydroxyl, epoxy or oxy groups. In carotenoids, the polyene chain with conjugated double bonds is responsible for their characteristic absorption spectra and specific photochemical properties. Among the carotenes, natural β-carotene is the precursor of vitamin A and has preventive action against eye diseases and cancer. Carotenes enhance immune response and protect skin cells against UV radiations. They help to lower the risk of cardiovascular diseases, age related vision disorders, asthma and reduce inflammation. Lycopene in red coloured tomatoes is effective against oxidative stress[21,22]. Along with carotene and lutein, it provides protection against lung, breast, uterus and prostate cancers. Green leafy vegetables and corn are best sources of xanthophylls and protect retinal part of human eye. Astaxanthin, a xanthophylls found in sea foods, and limonoids present in citrus fruits are biologically active phytochemicals which protect lung tissue from free oxygen radicals and inhibit proliferation of human breast cancer [6].

Tocopherols and Tocotrienols: They are non-polar constituents of biological membranes that exist in nature of lipid phase. Vitamin E is found in unrefined cereal grain, vegetable oils, wheat germ, nuts, fruits and green leafy vegetables and have beneficial effects in heart, cancer, cataract, and Alzheimer's disease. α-tocopherol is the most abundant forms of tocopherols. γ-tocopherols can reduce most effectively the concentration of nitrogen dioxide that is involved in carcinogenesis, arthritis, and neurologic diseases. The unique structure of α-tocopherol enables it to act as an effective antioxidant and to be regenerated through reaction with other oxidants. Tocopherols, mainly found in palm oil, cereal grains and kale are potential antioxidants and are associated with the reduced risk of cancer, Alzheimer's and cardiovascular diseases. They also have cholesterol lowering ability and inhibit LDL (Low Density Lipoprotein) oxidation. α-tocopherol is preferentially absorbed compared to its other forms. Even though tocotrienols have a higher radical scavenging activity than tocopherols, they are less bio-available as compared to the latter.

Ascorbic acid (Vitamin C): Rose hips, chillies, guava, citrus fruits, berries, kiwi fruit and some vegetables are main sources of vitamin C with beneficial effects in cardiovascular health, cancer, immunity and connective tissues. It is leading natural antioxidant that can scavenge ROS and has anticarcinogenic effect. It is excellent electron donor, which makes generation of relatively stable semidehydroascorbic acid as well as its easy conversion from dehydroascorbic acid to ascorbic acid possible. Synthetic antioxidants such as BHT and BHA were found less effective than ascorbic acid. Oxidation of ascorbic acid is highly influenced by heat, light, water, pH, oxygen concentration and metal ions like Cu+2 and Fe+3. It may be related to the prevention of some forms of cancer and heart diseases. Ascorbic acid and tocopherol supplementation can substantially reduce oxidative damage. Their effects are greater in non-smokers than smokers. Smoking induces oxidative stress from numerous free radical compounds in the gaseous phase and the radicals formed from ascorbic acid acts as pro-oxidant in smokers.

Lipoic acid: Some sulphur containing compounds like GSH [glutathione], lipoic acid and dihydro liopic acid present in spinach, broccoli and yeast show antioxidant activities. They prevent oxidative damage of proteins, regenerate GSH in the liver, kidney and lung tissues, protect brain and nerve tissues, and reduce diabetes related complications and thus play an important role in reduction of blood glucose concentration. Lipoic acid improves mitochondrial membrane potential. Age related memory loss and brain ailments, including Alzheimer's and Parkinson's disease. It also has the ability for radical scavenging and metal chelation.

Polyphenols: The term polyphenol or phenolics refer precisely to those chemical compounds which have an aromatic ring with hydroxyl substituent (s)., including their derivatives. On the basis of chemical structure, they can be classified into phenolic acids, flavonoids, stibenes and lignans. Berries, ginkago, onions, apples grapes, chamomile, dandelion, green tea [48], hawthorn, licorice, rosemary, thyme, and some beverages (like red wine, coffee, cocoa, beer) are natural sources of polyphenols with strong antioxidant activity and biological properties. They can enhance the activity of vitamin C. they act against allergies, ulcers, tumours, platelet aggregation, are and are also effective in controlling hyper tension. Flavonoids possess ideal structure for free radical scavenging activity and have been found to be more effective antioxidant in vitro than tocopherols and ascorbates. More than 4,000 flavonoids have been identified in plants, which are responsible for the colour of vegetables, fruits, grains, seeds, leaves, flowers, bark and product derived from them. They are powerful antioxidant that inhibit the oxidation of low density lipoprotein (LDL), a major factor in the promotion of atherosclerosis, which is the plaque build-up in arteries that can lead to heart attack or stroke. Isoflavones like genestein [13] and daidzein found abundantly in legumes such as lentils, chickpeas and

soyabeans, have neutraceutical properties against tumour growth and cancer and they form one of the main classes of oestrogenic substances in plants.

Polyphenols are powerful scavengers of free radicals and also act as anti-inflammatory, anti-ulcer, antitumour and anticancer agents. They act as potent chain-breaking antioxidants and possess vitamin C stabilising activity by increasing its adsorption. Their therapeutic usefulness has been demonstrated in gastrointestinal haemorrhages, radiation reactions, erythroblastosis, menorrhagia, bleeding cystilis, tuberculosis, haemophysis, periodontal diseases, epitasis, and ophthalmic disorders. Polyphenols bind with transition metals, particularly iron and copper, and thus inhibit transition metal-catalysed free-radical formation. The chelated transition metals become unavailable to interact with other compounds and initiate biologically damaging reactions. Polyphenols inhibit lipid peroxidation, oxidation of linoleic acid and Fe+2 catalysed oxidation of glutamine synthase, through free radical scavenging and removal of metals ions from catalytic sites via chelation. They are also known to modify the activities of some enzymes involved in immune functions, carcinogenesis, cellular transformations, tumour growth and metastasis. Biological effects of phenols are of great interest since evidence has been found that they offer protection against several diseases. They have the potential to inhibit oxidation of LDL that is considered to be a key mechanism in atherosclerosis. Certain studies have shown that the consumption of foods rich in polyphenols results in reduced susceptibility of LDL to oxidation and are also effective scavengers of free radicals, responsible for DNA damage and tumour promotion. They were found to have beneficial effect in rheumatoid arthritis and experimental studies showed their anti-inflammatory activity.

Epidemiological studies provide convincing evidence that a diet rich in antioxidant is associated with a lower incidence of degenerative diseases. Cereals, legumes (barley, corn, nuts, oats, rice, sorghum, wheat, beans, and pulses), oilseeds (rapeseed, canola, flax seed and olive seeds), fruits, vegetables and beverages (fruit juices, tea, coffee, cocoa, beer and wine) are the main sources of dietary polyphenols. Fruits like apple, grape, pear, cherry and various berries contain up to 200-300mg polyphenols per 100 g fresh weight. A glass of red wine or a cup of coffee or tea contains about 100mg polyphenols. Their total dietary intake may be about 1g per day, which is about 10x higher than of vitamin C and 100 times higher than those of vitamin E and carotenoids. The major constituents of tea polyphenols of tea polyphenols constitute up to 30 per cent of the dry weight of green leaves and 9-10% of dry weight of black tea leaves. Citrus fruits are main sources of flavomones, and hesperidin is found in abundance (120-250mg/l) in orange juice. Fruits, particulary onions are rich source of quercetin. Anthocyanins are pigments of fruits such as cherries, plums, strawberries and red currant, ranging from 0.15 to 4.5 mg/g in fresh berries. Soyabean is main source of isoflavonoids like genistein and daidzein that have important role in prevention of cancer and osteoporosis. People who consume traditional diets rich in soy and tea rarely have breast, uterus and prostate cancer [55].

Bitter ways to slow aging: Cumin or jeera, extensively used in Indian cuisine, is known to possess antiparasitic and antimicrobial properties. It is also used to cure fewer and as a painkiller. One of the variants of cumin, bitter cumin (kalijiri), has been studied for its antianagsic and astringent properties. It is dried seed of the herb Centatherum anthelminticum and used to treat a wide range of diseases from vitiligo to hyperglycemia. Now, a research suggests that bitter cumin contains high levels of antioxidants. ROSs, also known as free radicals, are produced as part of the metabolic processes necessary for life. These are required or various functions like cell growth and energy production. But conversely their increased concentrations and non-removal from of the body can lead to abnormalities like neurodegerative disorders and cancer. Anti oxidants detoxify these free radicals and help in their removal from the body. By neutralising these ROS, antioxidants also slow down the aging process. Common antioxidantsinclude vitamin C and E.

Researchers at the CFTRI (Central Food Technological Research Institute), Mysore, conducted an experiment on bitter cumin treated with a combination of CH3COCH3 (actone), CH3OH (methanol) and H2O (water). the antioxidant activity of bitter cumin extracts were then characterised using various free radical scavenging tools like DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid). To validate the results, extracts were also tested for their reducing power –ability to donate electrons. Higher the reducing power of the sample better is the antioxidant activity. The results revealed that bitter cumin extracts were strong antioxidants with different magnitude of potency in scavenging different ROS at the μg concentration. The phenol extract of bitter cumin contains an array of phenolic compounds which may be responsible for its antioxidant activity. The extracts were also strong electron donors and hence potential reducing agents. Another marker of antioxidation, he adds. Bitter cumin extracts were also able to minimize oxidative damage to DNA, one of the most detrimental effects of free radicals. They also found that radical scavenging activity of bitter cumin phenols is the highest among all plant phenols. Previous studies have reported number, type and concentrations of phenols in plants exhibit extreme diversity. IT has been observed on broad spectrum analysis, reported phenolic compounds, antioxidant, anto-hyperglycemic, antimicrobial activity of bitter

cumin. It is a native to the Upper Egypt but now grown in countries across the world especially India, North Africa, China and the US.

II. Therapeutic Significance of Soyabean

Soybean 40% protein and 20% oil and thus, assumes a predominant position in solving the problem of food shortage the world over. A native of China, soybean has been cultivated for food well over 13,000 years. The Chinese name for soybean means 'greater bean'. Like other beans, soybeans grow in pods, containing edible seeds (Figure 1). While we most often think of them as being green, the seeds can also be yellow, brown or black.

Figure 1: Exotic Variety of Soybean

Today soybeans are grown all over the world. This plant was introduced in most countries as a source of oil food and protein for livestock but now it is commercially grown for many food and industrial purposes. About 70% of the total production goes for oil extraction and rest for seed purposes (10%) and direct food uses (20%). The oil so obtained is refined and used for culinary purposes. It is also used as an important ingredient for industrial products such as paints, plastics, lubricants and bio-fuels. The main by-product of the oil industry, namely lecithin (phospholipid) finds commercial application as a nutritional supplement and emulsifier. Other by-product includes hulls, which are used in animal feeds and as a source of fiber [23]. The meal primarily used as a source of protein for poultry, piggery, livestock, aquaculture, etc. Soy meal has more than 50% edible grade protein, which can also be diverted for food uses. However the meal from the solvent extraction plants must be made edible grade and devoid of the residual solvents, which may cause various physiological disorders in humans. The ISO (International Standard Organisation) recommends 50ppm of residual hexane while BIS (Bureau of Indian Standards) allows 170ppm of such residual solvents. In an innovative process, developed by the INTSOY (International Soybean Center) at the University of Illinois, the soya bean oil is extracted primarily through extrusion. The meal is devoid of the solvent and also contains low profile of fat. It may be used for direct food uses through either supplementation of fortification with traditional foods.

III. Major Constituents of Soy

Soy foods contain all nine essential amino acids. The PDCAASW (protein digestibility corrected amino acid score) is 1.0, which is equivalent to animal protein. Soybeans have a number of nutritional advantages over other food legumes. Soybean derives about 35 to 38% of its calories from protein compared to ~20 to 30% in other legumes. It contains on an average 40% protein that is much higher when compared to other legumes. The values for other legumes are: chickpea, 4.9-29.6%; peas, 21.2-32.9%; cowpea, 20.9-38.5; pigeon pea, 18.8-28.5%; green gram, 20.8-33.1%; lentil, 20.4-30.5% and lathyrus, 22.7-29.6%. The quality of soya proteins is almost at par with egg or milk proteins, which are ideal with reference to their essential amino acid make-up24. Like other pulses, soybeans are deficient in sulphur-containing amino acids such as cystine and methionine. Since it has more lysine than cereals, its blending with them makes the product well balanced. It is therefore suggested to blend soybean with cereals, millets and other pulses at different proportions as per our body requirement. Substitution of soy protein for a protein source of animal origin can result in reduction of calorie. Soy protein concentrate and soy protein isolate are reported to have 330 cal/100g. The soy-based diet thus lowers the incidences of obesity. Active isoflavone compounds found in soy, specifically, genistein [24] help us stay lean by producing fewer and smaller fat cells.

Approximately 40% of the calories in soybeans are derived from fats. Soybean has exceptionally large quantities of fat. It has on an average 20% oil. The oil is hypocholesterolemic. The oil content is much higher than other pulses such as black gram, 1.64%; pigeon pea, 2.19%; cowpea, 2.05%; chick pea, 4.99%; lentil, 1.17%; lathyrus, 1.0% and green gram, 2.14%. The quality of oil is normally judged by its fatty acid composition. More the unsaturated fatty acids better the quality of oil. It contains about 78% of unsaturated fatty acids. Out of them linoleic and linolenic constitute 58%. They are called PUFS (polyunsaturated fatty acids). The IV (iodine value) is in the range of 125-135. The oil remains liquid over a relatively wide range. The oil can be hydrogenated selectively for blending with semi solid or liquid oils. Naturally occurring antioxidants/ tocopherols are present and are not completely removed during processing. However, soya oil has certain disadvantage like high phosphate content (2%), which must be removed by processing. The oil also contains 7-8% linolenic acid, which is responsible for flavour and odor reversion [25.

It contains about 20-30% carbohydrates. Mostly they are galacto oligosaccharides such as raffinose,

stachyose and verbascose. It has little starch. The carbohydrate content is much lower than the other legumes where the major portion is starch such as black gram, 56.5-63.7%; chickpea, 60.1-61.7; green gram, 53.3-61.2; pigeon pea, 57.3-58.7% and lentil, 59.7-61.0%. It also has considerable amounts of calcium (226mg); phosphorus (546mg); iron (8.5mg); iron (8.5mg); magnesium (236mg); copper (2.4mg); and sodium (27.9mg)/ 100g of beans. All whole, unprocessed plant foods contain dietary fiber. One serving of soybeans provides approximately 8 grams of dietary fiber. However, many soya foods are processed in ways that decrease their fiber content significantly. Tofu and soya milk, two of the more popular soya foods, contain very little fiber. Soya foods that utilize the whole bean such as tempeh, soya flour and textured soya protein, are high in fiber. About 30% of the fiber in soya foods is soluble fiber [26].

It is a rich source of vitamin A (426mg); thiamine (73mg); riboflavin (39mg) and niacin (3.2mg)/ 100g beans. Soya foods are the richest source of isoflavones. These are phyto-serms (selective estrogen receptor modulators). They have some estrogen-like qualities and have non-hormonal properties as well. The two primary isoflavones in soybeans are genistein and diadzein and their glycosides. They contribute to many protective effects. Soy foods and other soy based dairy analogues can serve as a balanced and remedial substitute of dairy milk for lactose intolerant persons. This condition arises mainly due to lack of beta galactosidase, the enzyme responsible for the hydrolysis of lactose in the intestine. The lactose is in turn degraded by the colonic bacteria into acid and carbon dioxide causing gastric discomfort such as flatulence, bloating, belching and diarrhoea. Since soybean has no lactose in it, the products prepared from soybean, namely, soy paneer and other soymilk analogues can serve as an ideal substitute of regular milk. There is some evidence that soya foods may help with sugar control in diabetics. Soy may also help lower risk of some of the complications of diabetes, such as kidney disease. Soybeans have a very low GI (glycemic index) and are valuable in a diabetic diet. Blood sugar control may also be improved by choosing carbohydrates that are high in soluble fiber. It helps in the slow absorption of the sugars. In kidney disease, a soy-based diet may be preferable to the traditional low protein diet from decreasing the renal damage. Soy provides high quality protein without stimulating hyper filtration and proteinuria. It prevents kidney damage by lowering serum LDL cholesterol levels [27].

Soybeans have a nutrient profile for heart health and have other properties that may help lower risk for heart disease. Soy protein lowers the total and LDL cholesterol levels. Soy foods are excellent choice for a heart-healthy diet. Soy oil provides the plant-derived omega-3 fatty acid, ALA, while fish oil contains the marine-derived omega-3 fatty acids, EPA and DHA. These omega-3 fatty acids improve heart function by providing greater variability between beats, therefore reducing the risk of arrhythmia and/ or sudden death. Soy in the diets will have significant reduction in both diastolic and systolic blood pressure. Not only the total blood cholesterol is significantly lowered, the level of HDL(High Density Lipoproten) good cholesterol are also significantly increased. Soy protein can reduce high blood cholesterol levels by 10 to 15% - enough to cut the chances of a heart attack by upto 30%. Soy protein inhibits cholesterol oxidation. Oxidised cholesterol is cholesterol that has undergone structural changes because of exposure to oxygen, damage arteries.

The hormonal changes that occur during menopause can cause a variety of symptoms and increase risk for heart disease and osteoporosis. During perimanopause women experience fluctuations in estrogen levels. This can cause hot flashes, night sweats, insomnia, vaginal dryness or headaches. HRT (Hormonal Replacement Therapy) is commonly prescribed to help prevent the negative health effects of menopause. However, many women do not want to take HRT because of the possible increased risk for breast cancer. Soya foods, which contain isoflavones, may decrease the health risk associated with menopause. They also lower the rates of osteoporosis and heart disease leading to longer expectancy. Soya foods contain anticarcinogens that may prove protective. They lower the incidences of breast, prostate and colon cancers. Soy foods fit in the formulation of a health promoting diet. The fiber in soyabeans also provides preventive therapy for several other conditions. High-Fiber soybeans may be able to help reduce the risk of colon cancer. As a matter of fact, in areas of the world where soybeans are eaten regularly, rates of colon cancer, as well as some other cancers, including breast cancer, tend to be low. Soybean fiber may also be able to reduce the symptoms of diarrhoea or constipation in suffers o irritable bowel syndrome [28].

IV. Therapeutic Significance of Aloe Vera

Aloe vera (Figure 2) contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids. It contains vitamins A (β-carotene), C and E, which are antioxidants. It also contains vitamin B12, folic acid, and choline.

Figure 2 : Exotic Variety of Aloevera

Antioxidant neutralizes free radicals. It contains 8 enzymes: aliiase, alkaline phosphatase, amylase, bradykinase, carboxypeptidase, catalase, cellulase, lipase, and peroxidase. Bradykinase helps to reduce excessive inflammation when applied to the skin topically, while others help in the breakdown of sugars and fats. It provides Ca (calcium), Cr (chromium), Cu (copper), Se (selenium), Mg (magnesium), Mn (manganese), K (potassium), Na (sodium) and Zn (zinc). They are essential for the proper functioning of various enzyme systems in different metabolic pathways and few are antioxidants. It provides monosaccharides (glucose and fructose) and polysaccharides: (glucomannans/polymannose). These are derived from the mucilage layer of the plant and are known as mucopolysaccharides. The most prominent monosaccharide is mannose-6-phosphate, and the most common polysaccharides are called glucomannans [β-(1, 4)-acetylated mannan]. Acemannan, a prominent glucomannan has also been found. Recently, a glycoprotein with antiallergic properties, called alprogen and novel anti-inflammatory compound, C-glucosyl chromone, has been isolated from Aloe vera gel. It provides 12 anthraquinones, which are phenolic compounds traditionally known as laxatives. Aloin and emodin act as analgesics, antibacterials and antivirals. It provides 4 plant steroids; cholesterol, campesterol, β-sisosterol and lupeol. All these have anti-inflammatory action and lupeol also possesses antiseptic and analgesic properties. Auxins and gibberellins that help in wound healing and have anti-inflammatory action.

It provides 20 of the 22 human required amino acids and 7 of the 8 essential amino acids. It also contains salicylic acid that possesses anti-inflammatory and antibacterial properties. Lignin, an inert substance, when included in topical preparations, enhances penetrative effect of the other ingredients into the skin. Saponins that are the soapy substances form about 3% of the gel and have cleansing and antiseptic properties. Glucomannan, a mannose-rich polysaccharide, and gibberellin, a growth hormone, interacts with growth factor receptors on the fibroblast, thereby stimulating its activity and proliferation, which in turn significantly increases collagen synthesis after topical and oral Aloe vera [9]. Aloe gel not only increased collagen content of the wound but also changed collagen composition (more type III) and increased the degree of collagen cross linking. Due to this, it accelerated wound contraction and increased the breaking strength of resulting scar tissue. An increased synthesis of hyaluronic acid and dermatan sulphate in the granulation tissue of a healing wound following oral or topical treatment has been reported.

Aloe vera gel has been reported to have a protective effect against radiation damage to the skin. Exact role is not known, but following the administration of aloe vera gel, an antioxidant protein, metallothionein, is generated in the skin, which scavenges hydroxyl radicals and prevents suppression of superoxide dismutase and glutathione peroxidase in the skin. It reduces the production and release of skin keratinocyte-derived immunosuppressive cytokines such as interleukin-10 (IL-10) and hence prevents UV-induced suppression of delayed type hypersensitivity. Aloe vera inhibits the cyclooxygenase pathway and reduces prostaglandin E2 production from arachidonic acid. Recently, the novel anti-inflammatory compound called C-glucosyl chromone was isolated from gel extracts. Alprogen inhibit calcium influx into mast cells, thereby inhibiting the antigen-antibody-mediated release of histamine and leukotriene from mast cells.7In a study on mice that had previously been implanted with murine sarcoma cells, acemannan stimulates the synthesis and release of interleukin-1 (IL-1) and tumor necrosis factor from macrophages in mice, which in turn initiated an immune attack that resulted in necrosis and regression of the cancerous cells. Several low-molecular-weight compounds are also capable of inhibiting the release of reactive oxygen free radicals from activated human neutrophils. Anthraquinones present in latex are a potent laxative. It increases intestinal water content, stimulates mucus secretion and increases intestinal peristalsis.

Antiviral and antitumor actions may be due to indirect or direct effects. Indirect effect is due to stimulation of the immune system and direct effect is due to anthraquinones. The anthraquinone alone inactivates various enveloped viruses such as herpes simplex, varicella zoster and influenza. In recent studies, a polysaccharide fraction has shown to inhibit the binding of benzopyrene to primary rat hepatocytes, thereby preventing the formation of potentially cancer-initiating benzopyrene-DNA adducts. An induction of glutathione S-transferase and an inhibition of the tumor-promoting effects of phorbol myristic acetate has also been reported which suggest a possible benefit of using aloe gel in cancer chemoprevention. Mucopolysaccharides help in binding moisture into the skin. Aloe stimulates fibroblast which produces the collagen and elastin fibers making the skin more elastic and less wrinkled. It also has cohesive effects on the superficial flaking epidermal cells by sticking them together, which softens the skin. The amino acids also soften hardened skin cells and zinc acts as an

astringent to tighten pores. Its moisturizing effects has also been studied in treatment of dry skin associated with occupational exposure where aloevera gel gloves improved the skin integrity, decreases appearance of fine wrinkle and decreases erythema. It also has anti-acne effect. Aloe vera contains 6 antiseptic agents: Lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and S (sulphur). They all have inhibitory action on fungi, bacteria and viruses

V. Therapeutic Significance of Spirulina

Spirulina is a blue-green algae (cyanobacterium). It is a simple, one-celled form of algae that thrives in warm, alkaline fresh-water bodies. The name "spirulina" is derived from the Latin word for "helix" or "spiral"; denoting the physical configuration of the organism when it forms swirling, microscopic strands. Spirulina (Figure 3) is being developed as the "food of the future" because of its amazing ability to synthesize high-quality concentrated food more efficiently than any other algae. Most notably, Spirulina is 65 to 71 % complete protein, with all essential amino acids in perfect balance. In comparison, beef is only 22 % protein. Spirulina has a photosynthetic conversion rate of 8 to 10 %, compared to only 3 % in such land-growing plants as soybeans. In addition, Spirulina is one of the few plant sources of vitamin B12, usually found only in animal tissues. A teaspoon of Spirulina supplies 212x the RDA (Recommended Daily Allowance) of vitamin B12 and contains over twice the amount of this vitamin found in an equivalent serving of live [29].

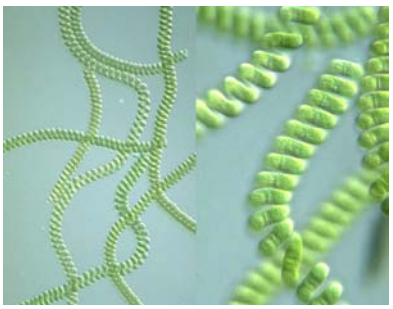

Figure 3 : Exotic Variety of Spirulina

Spirulina also provides high concentrations of many other nutrients - amino acids, chelated minerals, pigmentations, rhamnose sugars (complex natural plant sugars), trace elements, enzymes - that are in an easily assimilable form. Even though it is single-celled, Spirulina is relatively large, attaining sizes of 0.5mm in length. This is about 100x the size of most other algae, which makes some individual Spirulina cells visible to the naked eye. Furthermore, the prolific reproductive capacity of the cells and their proclivity to adhere in colonies makes Spirulina a large and easily gathered plant mass. The algae are differentiated according to predominating colorations, and are divided into blue-green, green, red and brown. Spirulina is one of the blue-green algae due to the presence of both chlorophyll (green) and phycocyanin (blue) pigments in its cellular structure. Even though Spirulina is distantly related to the kelp algae, it is not a sea plant. However, the fresh-water ponds and lakes it favors are notably more alkaline - in the range of 8 to 11 pH than ordinary lakes and cannot sustain any other forms of microorganisms. In addition, Spirulina thrives in very warm waters of 32 to 45° C (~ 85 to 112°F, and has even survived in temperatures of 60° C (140°F) [30].

Certain desert-adapted species will survive when their pond habitats evaporate in the intense sun, drying to a dormant state on rocks as hot as 70°C (160°F). In this dormant condition, the naturally blue-green algae turns a frosted white and develops a sweet flavor as its 71 % protein structure is transformed into polysaccharide sugars by the heat. Some scientists speculate that the "manna" of the wandering Israelites, which appeared miraculously on rocks following a devastating dry spell and was described as tasting "like wafers made with hone" may have been a form of dried, dormant Spirulina. This ability of Spirulina to grow in hot and alkaline environments ensures its hygienic status, as no other organisms can survive to pollute the waters in which this algae thrives. Unlike the stereotypical association of microorganisms with "germs" and "scum", Spirulina is in fact one of the cleanest, most naturally sterile foods found in nature. Its adaptation to heat also assures that Spirulina retains its nutritional value when subject to high temperatures during processing and shelf storage, unlike many plant foods that rapidly deteriorate at high temperatures.

Spirulina is also unusual among algae because it is a "nuclear plant" meaning it is on the developmental cusp between plants and animals. It is considered somewhat above plants because it does not have the hard cellulose membranes characteristic of plant cells, nor does it have a well-defined nucleus. Yet its metabolic system is based on photosynthesis, a process of direct food energy production utilizing sunlight and chlorophyll, which is typical of plant life forms. In essence, Spirulina straddles that fork in evolutionary development when the plant and animal kingdoms differentiated. Thus it embodies the simplest form of life. In contrast, other algae such as Chlorella have developed the hard indigestible walls characteristic of plants. Recent advances in biochemistry and molecularly biology techniques provide new, powerful tools for studying the antioxidant enzymes and for elucidating the mechanisms of the actions of antioxidants. Thus the future of antioxidants hold promise to ensure a better, disease-free lifestyle for mankind by scavenging free radicals and consequently preventing mutagenic changes and associated disorders[31].

VI. Therapeutic Significance of Gooseberry (Aaonla/Amla)

The medicinal, culinary, cosmetics, aromatic and sacred applications of plants ware well known to Ayurveda practitioners. Gooseberry, Indian gooseberry, is such potent gift of nature to humankind. It contributes toward health and longevity and is an indispensable part of Ayrvedic and Unani system of medicine. Scientific name of this tree is Emblica officinalis. It is referred to in ancient text as the best medicine to prevent aging.

VII. Constituents & Applications of Gooseberry

One of the most popular vitamins prominent in most skin care products is ascorbic acid (vitamin C). Gooseberry contains 20x the amount of vitamin C found in oranges. This anti aging vitamin has been studied and confirmed as being an extremely effective addition to skin care routines as it is necessary for the synthesis of inter-cellular cement 'collagen'. Collagen is produced by the skin naturally and no creams or lotions can replace collagen. External application of collagen has absolutely no effect on the skin. Our skin doesn't have the ability to absorb collagen; it can only produce the same naturally. Collagen is responsible for maintaining the skin's elasticity; it keeps the skin supple and prevents cell degeneration which is the main cause of aging. When antioxidant vitamin C is added to skin, it helps our skin get rid of free radicals. Since free radicals can greatly damage our skin, the use of vitamin C is vital to our skin cells health. Vitamin C also helps to break up dead skin cells to reveal a smooth, bright complexion.

Figure 4 : Exotic Variety of Gooseberries

It has now been reported that people who eat plenty of vitamin C-rich food have fewer wrinkles than people whose diet contained little of it. Relative to this, they also observed that if Gooseberry is taken regularly as dietary supplement, it counter acts the toxic effects of prolonged exposure of environmental heavy metals like Pb (lead), Al (aluminium) and Ni (Nickel) which cause environmental damages globally especially as researchers cautioned that when Gooseberry is dried in shade then much of the vitamin C is retained, to get the maximum out of Gooseberry it should be taken raw with little salt.

According to ancient Indian Ayurvedic principles, Gooseberry has the ability to rejuvenate not only skin but also the heart and bones. Since free radicals can greatly damage our skin, the use of vitamin C is vital to our skin health. Vitamin C also helps to break up dead cells to reveal a smooth, bright complexion. Researchers now report that people who eat plenty of vitamin C-rich food have fewer wrinkles than people whose diet contained little of it. Relative to this, they also observed that if Gooseberry is taken regularly as dietary supplement, it counteracts the toxic effect of prolonged exposure to environmental heavy metals like lead, aluminium and nickel which cause environmental damages globally especially as researchers cautioned that when Gooseberry is dried in the shade then much of the vitamin C is retained. To get the maximum out of Gooseberry it should be taken raw with very little salt. It is often used in the form of pickles and is dried and powered. It is used in general vitality tonics. Gooseberry is low in sugar and high in fibre which is yet benefit of Gooseberry. It also aids metabolism.

Gooseberry contains 720 mg of vitamin C/ 100 g of fresh fruit pulp, or up to 900 mg per 100 g of pressured juice which is required for good vision and mental development. It also aids metabolism. Gooseberry contains 720 mg of vitamin C/ 100 g of fresh fruit pulp, or up to 900 mg/ 100 g of pressed juice which is required for good vision and mental development. Gooseberry contains gallic acid, tannic acid, albumin, cellulose and minerals. Due to tannins, even dried form retains most of the vitamin content. Gooseberry normalizes body function, balances the neuro-endocrine system and improves immunity. Gooseberry normalizes body function, balances the neuro-endocrine system and improves immunity. Gooseberry's hair tonic is one of the best-kept secrets of Indian beauty, and it's one of the ways women keep their hair so shiny and strong (aside from fabulous genetics, of course).

Hair tonic is one of the best-kept secrets of Indian beauty, and it's one of the ways women keep their hair so shiny and strong (aside from fabulous genetics, of course). Indian gooseberry is an accepted hair tonic in traditional recipes for enriching hair growth and pigmentation. The Gooseberry, cut into pieces is dried preferably in the shade. These pieces are boiled in coconut oil till the solid matter becomes charred. This darkish oil is excellent in preventing greying. The water in which Gooseberry pieces are soaked overnight is also nourishing to hair and can be used for the rinse while washing the hair. Gooseberry is believed to enhance hair growth by stimulating the scalp, so it's often recommended for women suffering from thinning hair. It's also said to enhance wave and curl. For use as a scalp massage oil or deep conditioner, mix powdered Gooseberry with coconut or sesame oil.

To add volume, mix the powder with water to make a paste to the consistency of yogurt and let it sit for about 15 minutes to allow the powder to dissolve. To add volume, mix the powder with enough water to make a paste to the consistency of yoghurt and let it sit about 15 minutes to allow the powder to dissolve. Apply it to hair; let it soak in for a few minutes and then rinse. It is often used in the form of pickles and it is dried and powdered. The berry may also be used as vegetable. It is boiled in a small amount of water till soft and taken with a little salt. Let it soak in for a few minutes and then rinse. It stops hair loss and encourages nail and hair growth. It is used in general vitality tonics. It is also used in Trifla powder. It can be mixed with henna, basil and other herbs and be applied in hair in paste form. It is also used in trifla powder. It can be mixed with henna, basil and other herbs and applied in hair in paste form. This cures hair fall, hair greying. It dyes, beautifies hair and rids numerous hair ailments.

Gooseberry oil is one of the world's oldest natural hair conditioners. As an Indian herb, Gooseberry oil has been used since a very long time. It is used as hair oil basically for its cooling effect. It instantly penetrates the cuticle and fills it out. It moisturizes and hydrates the hair which adds volume naturally. It can also restore total shine and manageability without chemicals leaving the hair soft and renewed. It provides nourishment to hair roots, improves blood circulation in the scalp and will instantly stop premature greying and hair loss. It has s a host of antibacterial and antifungal activities thus eliminating dandruff in the scalp and psoriasis. In India, it was known as miracle fruit. According to 5000 year Indian Myth, it was considered as the nectar of the Gods because of the way it magically makes hair grows thicker, stronger and more manageable.

Indian gooseberry is beneficial in the treatment of respiratory disorders. It is especially valuable in tuberculosis of the lungs, asthma and bronchitis. Gooseberry, due to its high vitamin C content, is effective in controlling diabetes. A tablespoon of its juice mixed with a cup of bitter gourd juice, taken daily for two months will stimulate the pancreas and enable them to secrete insulin, thus reducing the blood sugar in the diabetes. Diet restrictions should be strictly observed while taking this medicine. It will also prevent eye complication in diabetes. Indian gooseberry is considered an effective remedy for heart disease. It tones up the functions of the organs of the body and builds up health by destroying the heterogeneous or harmful and disease causing elements. It also renews energy and possesses revitalizing effects.

The juice of Indian gooseberry with honey is useful in preventing eyesight. It is beneficial in the treatment of conjunctivitis and glaucoma, it reduces intraocular tension in a remarkable manner. A cup of juice with honey can be taken twice daily for this condition. To treat rheumatism a teaspoon of the powder of dry fruit mixed with 2 teaspoons of jaggery can be taken daily for two months. As an extremely rich source of vitamin C, Indian gooseberry is one of the best remedy for scurvy. Powder of this fruit, mixed with an equal quantity of sugar can be taken in doses of 1 teaspoon, thrice daily with milk. It has a host of antibacterial and antifungal activities thus dandruff in the scalp and psoriasis as well. In India, it was known as miracle fruit. According to 5000 year old Indian Myth, it was considered as the nectar of the Gods because of the way it magically makes hair grows thicker, stronger and more manageable. Indian gooseberry is beneficial in the treatment of respiratory disorders. It is especially valuable in tuberculosis of the lungs, it strengths the lungs, helping to fight chronic lung problems as well as upper respiratory infections.

Gooseberry leaves are useful in ophthalmic and incipient blindness. People use the fresh leaf juice of Gooseberry for wound dressing. According to traditional healers the fresh leaf juice is good hair tonic and they also used the leaves in hair tonic like its fruits. This combination is a boon for leprosy patients. The application increases the rate of healing. The application increases the rate of healing. Gooseberry root and bark are used in scorpion bite. Gooseberry seeds are acrid, and useful in treatment of asthma, bronchitis, leucorrhoea, etc. Many healers use Gooseberry seeds in treatment of diabetes. The seeds are also used in treatment of Epistaxis. The seed powder mixed with honey is considered as good for gynaecological troubles especially in case of leucorrhoea. In case of vomiting, the traditional healers recommended it with common herb Lal Chandan (Pterocarpus santalinus). Fresh leaves are eaten in combination with fresh curd or whey to treat stomach related diseases and diarrhoea. The traditional healers use the leaves in different ways. For treatment of Epistaxis, they apply the fresh leaf juice with camphor on head [35].

VIII. Therapeutic Significance of Broccoli

As the growing industrialization is continuously spitting out carcinogens into our environment and cancer of various hues are spreading their tentacles to take an ever increasing toll on human life, an apparent relief has been discovered in lowly fruits and vegetables rich in antioxidants, polyphenols and other cancer preventive chemicals. Among these, Italian broccoli, which is simply called as broccoli, a vegetable belonging to the Brassicaceae family has proved to be a most potent one. Besides, the unique anti-cancer and other medicinal properties, it is also rich in various nutrients, (depicted in table) that can ensure sound health and long life. Particularly, its vitamin C content is very high. One hundred grams of broccoli contains enough vitamin C to meet the daily requirement of an

adult person. This cool-weather crop is rich in vitamin C, folic acid and water soluble dietary fibres. It contains a number of nutrients with potent anti-cancer properties including diindolyl methane and selenium (Se). Particularly, 3, 3' – diindolyl methane is an active modulator of the innate immune response system with anti-viral, anti-bacterial and anti-cancer activities. Like other brassica vegetables broccoli is also rich in glucosinolates, which are metabolized to cancer preventive substances like isothiocyanates. Glucoraphanin, compound present in it can be processed into sulphoraphane, a well known anti-cancer agent. Broccoli leaf is edible and it contains a lot of β-carotene. Therefore, a high intake of broccoli has been found to reduce the risk of many types of cancer, especially prostate cancer. Recently, a research team from the NCI (National Cancer Institute) has found that eating broccoli and cauliflower once a week, decreases the aggressiveness of the disease by 45% to 52%. Similar effects have also been observed in case of colon cancer.

Figure 5 : Exotic Variety of Broccoli

Methods of storage and cooking have varying impacts on anti-cancer effects of broccoli. Domestic storage of the vegetable shows only minor loss of glucosinolate levels over 7 days. However, when stored at a much lower temperature the loss may be up to 33% by fracture of vegetable material during thawing. On the other hand, a total loss of 77% glucosinolate has been observed after boiling it for 30 minutes, but steaming for 2 minutes, microwave cooking for 3 minutes and stir-fry cooking for 5 minutes do not have any significant effects on those, except when the vegetable is finally shredded. Therefore, in order to derive the maximum benefits from broccoli the later three methods of cooking should be done with less water, which should be consumed along with the vegetables and the boiling time should also be reduced [60].

Nutritional constituents of Broccoli (per 100g of raw edible part); Energy = 30 kcal					
Sr No.	Constituents	Quantity	Sr No.	Constituents	Quantity
1.	Carbohydrate	6.64 g	11.	Vitamin B6	0.175 mg
2.	Dietary Fibre	2.6 g	12.	Folate (Vit. B9)	63 μg
3.	Fat	0.37 g	13.	Ascorbic acid (Vit.) C	89.2 mg
4.	Protein	2.82 g	14.	Niacin (Vit. B3)	0.639 mg
5.	Water	89.30 g	15.	Iron	0.73 mg
6.	Vitamin A equiv.	31 μg	16.	Magnesium	21 mg
7.	β carotene	361 μg	17.	Calcium	47 mg
8.	Thiamine (Vit. B1)	0.071 mg	18.	Phosphorous	66 mg
9.	Riboflavin (Vit. B2)	0.117 mg	19	Potassium	316 mg
10.	Pantothenic acid (B5)	0.573 mg	20.	Zinc	0.41 mg
					Source: USDA Nutrient database

This cool-weather crop is rich in vitamin C, folic acid and soluble dietary fibres. It contains a number of nutrients with potent anti-cancer properties, including diindolyl methane and Se. Many healers use the Gooseberry seeds in treatment of diabetes. The seeds are also used in treatment of epistaxis. The seed powder mixed with honey is considered as good for gynaecological troubles especially in case of leucorrhoea. In case of vomiting, the traditional healers recommend it with common herb Lal Chandan (Pterocarpus santalinus). Fresh laves are eaten in combination with fresh curd or whey to treat stomach related diseases and diarrhea.for treatment of Epistaxis, the fresh leaf juice with camphor is applied on head.

IX. Phytochemical Significance of Garlic

Garlic has long been used throughout the world in cooking as well as in medicine. From the earliest times garlic has been used as a food. It formed part of the diet of the Israelites in Egypt and of the laborers employed by Khufu in constructing the pyramid. Garlic is still grown in Egypt, but the Syrian variety is the kind most esteemed now (Figure 1). It was consumed by the ancient Greek and Roman soldiers, sailors and rural classes and, according to Pliny the Elder by the African peasantry. Galen eulogizes it as the "rustic's theriac" and Alexander Neckam, a writer of the 12th century recommends it as a palliative of the heat of the sun in field labor. In his Natural History Pliny gives an exceedingly long list of scenarios in which it was considered beneficial. It has been valued as an

application in confluent smallpox, and some dropsies cured by it alone, were also found. Early in the 20th century, it was sometimes used in the treatment of pulmonary tuberculosis or phthisis.

a) Based on modern science, garlic exerts several therapeutic effects

Anti-platelet Cardiovascular disease; Anti-atherosclerotic: Restoration of endothelial function and suppression of LDL oxidation; Anti-tumour: Cancers; Anti-oxidative: Reducing the risk of cardiovascular disease, stroke, cancer and aging " Reducing the risk of dementia, creasing homocysteine, blood pressure, and increasing microcirculation, which are important in diabetes " Treatment of viral hepatitis and acute liver injury; Anti-bacterial: Effective anti-bacterial agent for pneumonia-resembling bacteria, oral bacteria and on bacterial isolates from infected wounds; Anti-hypertensive: Lowers blood pressure; Anti-thrombotic: Lowers

coagulation; Anti-fungal : Treatment of human systemic fungal infections and cryptococcal meningitis.

Figure 1 : Exotic Variety of Garlic with Clove

There are more than 60 varieties of Garlic grown throughout the world. Health science experts have linked longevity to Garlic consumption. Snow Mountain Garlic from J & K has been clinically established to be the world's best garlic in terms of purity and potency. J & K garlic is the most unique rare herb on earth as it can only be grown successfully into a full plant in the snow mountain of the Himalayas at 6,000 feet above sea level and where oxygen is much less. This species has the ability to survive in very little oxygen and in extremely cold environment – up to 10 C. When spring arrives, the melting snow provided more than adequate water for enhanced plant maturity. Thus, the garlic bulb contains water/liquid from the Himalayan snow in its purest natural form Because of its ability to increase plant vessel capillary action viz. to transport soil nutrients to the top most part of the plant for maximum growth, J & K garlic is observed to be a very efficient vessel dilator for improved blood vessel health and performance for human vital senses and organs. It is in this research for a period of 4 ½ years that J & K garlic is now cultivated for global consumption. For generation, people are not only using garlic because of its medicinal value, but traditionally, people have rubbed their bodies with it, buried it besides their bodies in coffin, worn it around their necks, draped it on household walls and even prayed to it. This great bulb has a lot of benefits, because no other plant has been held out for so long as a cure for so many human ailments. That's why garlic has been considered as the "Wonder Drug". Garlic has been used medicinally for many years for treating bites, tumours, ulcers, snakebite, wounds, headaches, heart diseases, cancer, pimples, measles and many more. It exhibits antioxidant activity, is good for skin, and contains flavonoids, which are good for heart and body.

Garlic contains a range of compounds including "Allicin", which is a pungent oily liquid that gives crushed garlic cloves their characteristic smell, and has been shown to be the antibacterial agent due to its active sulphur. Garlic shows antifungal and antiviral properties. Raw garlic is very smelly, so in order to reduce it smell, you can simply add it to your gravy, salad dressings, to soup, yummy pizza or just garnish it before serving or have it in your own style. In summary, the garlic and its supplements have long been consumed in many cultures as a natural remedy against a range of human illnesses including bacterial, viral and fungal infections, hypolipidemic, antiplatelet, antitumoral, regulating blood pressure, lowering blood sugars, cholesterols levels and providing procirculatory effects. It is fascinating to observe how ancient cultures came to the same conclusion about garlics action and efficacy as confirmed from modern science.

Recent literature has pointed towards significant biological activity of these trisulphides and tetrasulfides found in various Allium species suggesting that a wide range of effects are caused by polysulfides.The biological activity of these polysulphides may include combination of several different cellular signalling pathways. Therefore, further research is required to understand mechanism action of polysulphides. Due to anti oxidative effects of AGE (Aged Garlic Extract) may help in preventing cognitive decline by protecting neurons from eurotoxicity, apoptosis and thus it may be beneficial in preventing ischemia/reperfusion related neuronal death and improve learning and memory. The possibility of herb-drug interactions, safety and efficacy should be discussed with health care professionals, because slight negligence in this regard can cause serious clinical consequences. Various therapeutic applications of garlic are concisely below.

Garlic is most well-known for its antibacterial and antiviral properties. They help control bacterial, viral, fungal, yeast and worm infections. Fresh garlic is thought to play a role in preventing food poisoning by killing bacteria like E. coli, Salmonella enteritidis, etc. The chemical ajoene found in garlic may help treat fungal skin infections like ringworm and athlete's foot. The anti-clotting properties of ajoene found in garlic help in preventing the formation of blood clots in the body. Hence, it may also increase the risk of bleeding after surgery. Angiotensin II is a protein that helps our blood vessels contract thereby increasing the blood pressure. Allicin in garlic blocks the activity of angiotensin II and

helps in reducing blood pressure. The polysulphides present in garlic are converted into a gas called hydrogen sulphide by the red blood cells. Hydrogen sulphide dilates our blood vessels and helps control blood pressure. Garlic protects our heart against cardiovascular problems like heart attacks and atherosclerosis. This cardio-protective property can be attributed to various factors. With age, the arteries tend to lose their ability to stretch. Garlic may help reduce this and may also protect the heart from the damaging effects of free oxygen radicals. The sulphur-containing compounds of garlic also prevent our blood vessels from becoming blocked and slow the development of atherosclerosis (hardening of the arteries). The anti-clotting properties of ajoene help prevent clots from forming inside the blood vessels.

Garlic has the ability to moderately lower our blood triglycerides and total cholesterol and reduce arterial plaque formation. Garlic is known to have anti-inflammatory property. It can help the body fight against allergies. The anti-arthritic property of garlic is due to diallyl sulphide and thiacremonone. Garlic has been show to improve allergic airway inflammation (allergic rhinitis). Raw garlic juice may be used to immediately stop the itching due to rashes and bug bites. Daily use of garlic might reduce the frequency and number of colds. Its antibacterial properties help in treating throat irritations. Garlic may also reduce the severity of upper respiratory tract infections. Its benefits in disorders of the lungs like asthma, difficulty of breathing, etc. make it a priceless medicine. Its ability to promote expectoration makes it irreplaceable in chronic bronchitis.

Garlic increases insulin release and regulates blood sugar levels in diabetics. Applying fat dissolving garlic extracts to corns on the feet and warts on the hands is thought to improve these conditions. Daily intake of garlic has been found to lower risk of most types of cancer. This anti-cancer property is due to allyl sulphides found in garlic. PhIP, a type of HCS (heterocyclic amine), has been associated with increased incidence of breast cancer among women. According to studies, diallyl sulphide found in garlic inhibits the transformation of PhIP into carcinogens. Ferroportin is a protein which helps in iron absorption and release. Diallyl sulphides in garlic increase production of ferroportin and help improve iron metabolism. Garlic's aphrodisiac property is due to its ability to increase the circulation. Simply put some crushed garlic clove directly on the affected tooth can help relieve toothaches due to its antibacterial and analgesic properties. But be aware that it can be irritating to the gum. It's believed that obesity is a state of long-term low-grade inflammation. According to recent research, garlic may help to regulate the formation of fat cells in our body. Pre-adipocytes are converted into fat cells (adipocytes) through inflammatory system activity. The anti-inflammatory property of 1, 2-DT (1, 2-vinyldithiin) found in garlic may help inhibit this conversion. This may help prevent weight gain.

X. Recent Advances Concerning to Beneficial Applicability of Garlic [69-70]

- You can increase the health benefits you receive from garlic by letting it sit after you've chopped it or crushed it. If you give your chopped/crushed garlic time to sit before changing its temperature (through cooking) or its pH (through the addition of acidic food like lemon juice), it will give the alliinase enzymes in garlic an opportunity to work on behalf of your health. For example, in the absence of chopping or crushing, research has shown that just 60 seconds of immediate microwaving will cause garlic to lose some of its cancer-protective properties. Immediate boiling of whole, intact garlic will also lower these properties, as will immediate addition of a very low-acid ingredient like lemon juice.
- Some of garlic's unique components are most durable in food (versus processed extract) form. Allicin—one of garlic's most highly valued sulphur compounds—stays intact for only 2-16 hours at r. t. when it is present in purified (extracted) form. But when it's still inside of crushed garlic, allicin will stay viable for 2-1/2 days.
- Garlic may help improve your Fe (iron) metabolism. That's because the diallyl sulfides in garlic can help increase production of a protein called ferroportin. (Ferroportin is a protein that runs across the cell membrane, and it forms a passageway that allows stored iron to leave the cells and become available where it is needed.)
- In addition to being a good source of selenium, garlic may be a more reliable source as well. Garlic is what we call a "seleniferous" plant: it can uptake Se from the soil even when soil concentrations don'tt favor this uptake.
- The cardioprotective benefits of garlic may partly rest on the production of hydrogen sulphide (H2S) gas. Our RBCs (Red blood corpuscles) can take sulphur-containing molecules in garlic (called polysulphides) and use them to produce H2S. This H2S in turn can help our blood vessels expand and keep our blood pressure in check. Interestingly, some processed garlic extracts cannot be used by our red blood cells in the same way and do not seem to provide the same level of cardioprotection that is provided by garlic in food form.
- While still in its very early stages, research suggests that garlic consumption may actually help to regulate the number of fat cells that get formed in our body. 1, 2-DT (1,2-vinyldithiin) is one of the

unique sulfur compounds in garlic that has long been recognized as having anti-inflammatory properties. But only recently have researchers discovered that some of our fibroblastic cells (called "preadipocytes") only evolve into full-fledged fat cells (called "adipocytes") under certain metabolic circumstances involving inflammatory system activity. 1, 2-DT may be able to inhibit this conversion process. Since obesity is increasingly viewed by researchers as a chronic state of low-grade inflammation, the inflammation-related benefits of garlic's 1, 2-DT may eventually be extended into the clinical area of obesity.

XI. Conclusion

As the growing industrialization is continuously spitting out carcinogens into our environment and cancer of various hues are spreading their tentacles to take an ever-increasing toll on human life, a relief has been discovered in lowly fruits and vegetables rich in antioxidants, polyphenols and other cancer preventive chemicals. Oxidative damage is often the result of high levels of unhealthy free radicals in the body. Free radicals are unstable or highly reactive moieties that cause unhealthy effects to our body's metabolism. Getting enough antioxidants in our diet to promote healthier tissues is essential to reduce the unwanted effects of these free radicals. When antioxidant vitamin C is added to skin, it helps our skin get rid of free radicals. Since free radicals can greatly damage our skin, the use of vitamin C is vital to our skin cells health. Vitamin C also helps to break up dead skin cells to reveal a smooth, bright complexion. It's now reported that people who eat plenty of vitamin C-rich food have fewer wrinkles than people whose diet contained little of it.

Indian curries are incomplete without garlic – a simple ingredient with packed health benefits. It is very strong and bitter but adds an unbelievable flavour to the cuisine. Any description of garlic is incomplete without mentioning its medicinal values. This miracle herb Garlic has been used since time immemorial as a medicine to prevent or treat various diseases and conditions. Garlic has a variety of potent sulphur-containing compounds which are the reason for its characteristic pungent odour. Allicin, the vital compound among them, is known to have great anti-bacterial, anti-viral, anti-fungal and anti-oxidant properties. The benefits of allicin can be best garnered when it's finely chopped, minced or pureed and let sit for some time. Garlic is also a reliable source of Se. Allicin, along with other compounds like ajoene, alliin, etc. found in them also have an effect on the circulatory, digestive and immunological systems of our body and help in lowering blood pressure, detoxification, healing, etc. 100 grams of broccoli contains enough vitamin C to meet the daily requirement of an adult person Gooseberry contains 720 mg of vitamin C/ 100 g of fresh fruit pulp, or up to 900 mg/ 100 g of pressured juice which is required for good vision and mental development. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, β-carotene, and vitamin C. Additionally, Se, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body can't manufacture these micronutrients so they must be supplied in the diet.

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Colourimetric Assay of Atomoxetine Hydrochloride by Simple Aurum Coupling Reaction in Bulk and Tablet Dosage Form

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Abstract- Simple, rapid and sensitive spectrophotometric procedure was developed for the analysis of atomoxetine hydrochloride (ATH) in pure form as well as in pharmaceutical formulations. The method was based on the reaction of ATH with gold (III) chloride in the pH range 3.5-4.5 forming violet colored complex solution, showing absorption maxima at 550 nm. The linear plot indicates that Beer's law is obeyed in the range of 5 – 80 **µ**g/ml of atomoxetine hydrochloride. The molar absorptivity and Sandell's sensitivity are 3.77 x 103M and 0.0774 µg cm-2 respectively. The standard deviation of the method for ten determinations ATH is 9.9827 x 103. The correlation coefficient (r2) of the experimental data of the calibration plot is 0.9997. The effective range of concentration for accurate determination of ATH as ascertained from Ringbom's plot and it is 10 – 80 **µ**g/ml.

Keywords: *atomoxetine hydrochloride, spectrophoto-metric, ringbom's plot, pharmaceutical formulations.*

GJMR-B Classification : *NLMC Code: QV 786, WA 730*

Strictly as per the compliance and regulations of:

Colourimetric Assay of Atomoxetine Hydrochloride by Simple Aurum Coupling Reaction in Bulk and Tablet Dosage Form

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Abstract- Simple, rapid and sensitive spectrophotometric procedure was developed for the analysis of atomoxetine hydrochloride (ATH) in pure form as well as in pharmaceutical formulations. The method was based on the reaction of ATH with gold (III) chloride in the pH range 3.5-4.5 forming violet colored complex solution, showing absorption maxima at 550 nm. The linear plot indicates that Beer's law is obeyed in the range of 5 – 80 µg/ml of atomoxetine hydrochloride. The molar absorptivity and Sandell's sensitivity are 3.77 x 103M and 0.0774 µg cm-2 respectively. The standard deviation of the method for ten determinations ATH is 9.9827 x 103. The correlation coefficient (r2) of the experimental data of the calibration plot is 0.9997. The effective range of concentration for accurate determination of ATH as ascertained from Ringbom's plot and it is 10 – 80 µg/ml.

Keywords: atomoxetine hydrochloride, spectrophotometric, ringbom's plot, pharmaceutical formulations.

I. Introduction

(-)-N-Methyl-3-phenyl-3-(o-tolyloxy)-propylamine hydrochloride is atomoxetine hydrochloride (ATH). The molecular formula is C17H21NO•HCl, Its molecular weight is 291.82. The structure of automoxetin is as follows

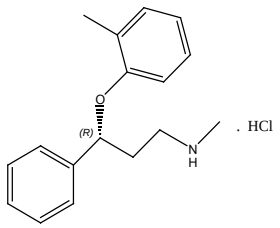

Figure 1 : Chemical Structure of atomoxetine hydrochloride

It is practically a white solid and has a solubility of 27.8 mg ml-1 in water. It is the first nonstimulant drug approved by the FDA for the treatment of attention-deficit hyperactivity disorder (ADHD) in children, adolescents and adults. ADHD is the most common neurobehavioral disorder among children with an estimated worldwide prevalence of 8–12%1.

ATH is not official in IP, BP, USP and EP. AMX is available commercially as capsules under brand name Straterra, Eli Lilly and company capsules. AMX capsules are intended for oral administration only. The capsules are available with strengths of 10, 18, 25, 40, 60 and 80 mg of ATH base. The capsules also contain pregelatinized starch and dimethicone.

A number of analytical methods based on liquid chromatography with fluorescence detection2, liquid chromatography/mass spectrometry/mass spectrometry 3-6 (LC/MS/MS) have been developed for the determination of atomoxetine in human plasma and urine. A chiral analytical method by using HPLC with UV7 has been reported for the determination of AMX impurities.

To the best of our knowledge, there is no work in the literature reported about the colourimetric method for the analysis of ATH in pharmaceutical formulations. Hence the author has made an attempt to develop simple and sensitive spectrophotometric method for the estimation of ATH in bulk drugs and in pharmaceutical formulations. The method was based on the reaction with gold(III) to form a violet coloured complex in the pH range 3.5 – 4.5.

II. Materials and Methods

All chemicals used were of analytical reagent grade and double distilled water was used for preparing the reagent solutions. ATH was obtained from Dr. Reddy's labs Hyderabad. Stock solution of ATH was freshly prepared by dissolving 100mg of ATH in 100mL of distilled water and then this was further diluted with distilled water so as to obtain working standard solution of 100 µg/mL.

a) Apparatus

All spectral and absorbance measurements were made on a Shimadzu UV-Visible digital Spectrophotometer (UV-160A) with 10mm matched quartz cells.

b) Pharmaceutical dosage form

Tablet label claim of 10 mg ATH (Straterra, Eli Lilly) was procured from local market.

c) Preparation of Atomoxetine hydrochloride solution

100 mg of ATH was weighed accurately and transferred into a 100ml standard flask, dissolved and made up to the mark with methanol. This solution is diluted as required.

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d) Preparation of Gold (III) solution

1gm of chloroauric acid (Johnson Mathews, materials technology, U.K.) is dissolved in distilled water after adding few drops dilute HCl. The solution is made upto the mark in 100 ml volumetric flask. The gold content of the solution is determined by rhodamine B method8. The working solutions are prepared by diluting the stock solution.

III. Experimental Method

a) Determination of Atomoxetine hydrochloride

To explore the possibility of employing the colour reaction for the determination of ATH, the absorbance of the experimental solution containing different amounts of ATH, keeping the Au (III) concentration constant is measured in the wavelength range 400 – 700 nm.

b) Determination of gold (III)

To explore the possibility of employing the colour reaction for the determination of gold(III) in trace level, the absorbance of the experimental solutions containing different amounts of gold(III), keeping the ATH concentration in excess, is measured in the wavelength range 400 – 700 nm.

c) Assay of Pharmaceutical dosage form of Atomoxetine hydrochloride

The present method for the determination Atomoxetine hydrochloride is applied for its determination in a pharmaceutical sample. A know aliquot of pharmaceutical sample solution of atomoxetine hydrochloride is added to a 10 ml volumetric flask containing 5ml of buffer solution of pH 4.0 and 0.5 ml of gold(III) (5.0 x 10-3M) solution 1.5 ml of 2% SDS solution. The contents are made upto the mark with distilled water. After heating for 60 minutes at 650C and cooling the solution to room temperature. The absorbance of the resulting solution is measured at 550 nm against the buffer blank. The amount of atomoxetine hydrochloride is computed from the predetermined calibration plot at 550 nm.

IV. Results and Discussion

The spectra presented in fig 2 show that the complex has an absorption maximum at 550 nm. Neither gold (III) nor atomoxetine hydrochloride have absorbance at 550 nm. Hence, analytical studies are made at 550 nm. However, in presence of excess atomoxetine hydrochloride the complex shows maximum absorbance at 550 nm.

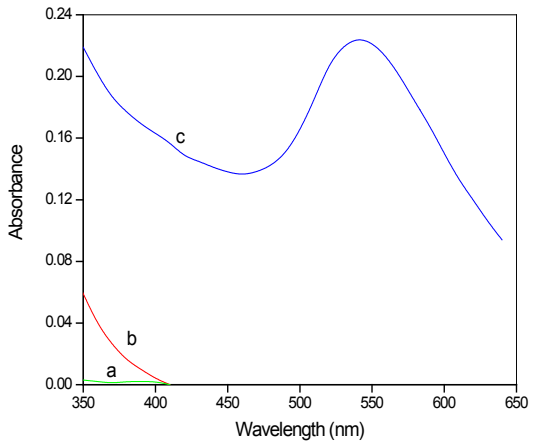

Figure 2 : Absorption spectra of AMX – Au(III) system
a. AMX Vs buffer blank
b. Au(III) Vs buffer blank
c. AMX – Au(III) Vs buffer blank
[Au(III)] = 5.0 x 10-3; [AMX] = 3.42 x 10-3M

a) Linearity

A plot of absorbance Vs amount of atomoxetine hydrochloride is presented in fig. 3 the straight line plot obtained obeys the equation A = 0.129C – 0.0009. The linear plot indicates that Beer's law is obeyed in the range of 5.0 – 80.0 μg/ml of atomoxetine hydrochloride.

The molar absorptivity and Sandell's sensitivity are 3.77 x 103 l mol-1 cm-1 and 0.0774 μg/cm2 respectively. The standard deviation of the method for ten determinations of 10 μg/ml of atomoxetine hydrochloride is 9.9826 x 10-4. The correlation coefficient (γ) of the experimental data of the calibration plot is 0.9997. The effective range of concentration for accurate determination of atomoxetine hydrochloride as ascertained from Ringbom's plot and it is 10.0 – 70.0 μg/ml.

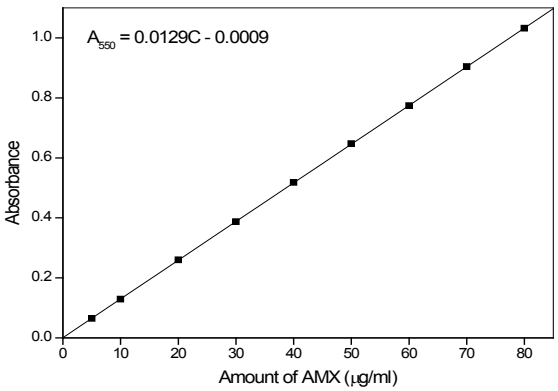

Figure 3 : Absorbance Vs amount of AMX (μg/ml)
[Au(III)] = 5.0 x 10-3M ; pH = 4.0 ; λ = 550 nm

b) Effect of excipients

Various amounts of excipients that are generally associated with the atomoxetine hydrochloride in its pharmaceutical formulations are added to a fixed amount of atomoxetine hydrochloride (10 μg/ml) solution and the absorbance measurements are carried

out under optimal conditions. The concentration (μg/ml) at which various ions do not cause an error of more than ± 4% in the absorbance is taken as the tolerance limit and the results are given in table 1.

Table 1 : Tolerance limit of excipients Amount of AMX = 10 μg/ml ; pH = 4.0

Excipient	Tolerance limit (μg/ml)
Fructose	1257
Glucose	901
Sucrose	1369
Lactose	1704
Gelatin	1811
Starch	1424
Sodium Alginate	1324
Boric acid	1891
Magnesium stearate	1576

The data in table1 indicate that the excipients that are associated with atomoxetine hydrochloride do not interfere even in large quantities in the determination of atomoxetine hydrochloride making the method highly selective and direct.

c) Assay of atomoxetine hydrochloride

The present method for the determination atomoxetine hydrochloride is applied for its determination in the tablet dosage form. The amount of atomoxetine hydrochloride is computed from the predetermined calibration plot at 550 nm. The results are presented in table 2

Table 2 : Assay of atomoxetine hydrochloride in pharmaceutical formulation

Sample (manufacturer formulation)	Label claim (mg)	Amount found * (mg)	Error (%)
Straterra, Eli Lilly and company	10.00	9.91	-0.90

* Average of seven determinations

Optimal characteristics, accuracy and precession, data of the determinations of atomoxetine hydrochloride and gold (III) are presented in table. 3.

Table 3 : Optimal and regression characteristics, precession and accuracy of the proposed method for atomoxetine hydrochloride and gold(III)

[AMX] = 3.42 x 10-3M ; pH = 4.0
[Au(III)] = 5.0 x 10-3M ; λ = 550 nm

Parameter	Atomoxetine hydrochloride	Gold(III)
Analytical wavelength (nm)	550	530
Beer's law limits (μg/ml)	5.0 - 80.0	9.84 – 157.42
Limits of detection (μg/ml)	2.2837	10.3723
Limits of quantization (μg/ml)	7.6170	34.7446
Molar absorptivity ($lmol^{-1}$ cm^{-1})	3.77 x 10^3M	0.75 x 10^3M
Sandell's sensitivity (μg cm^{-2})	0.0774	0.2625
Regression equation (y = a + bx)		
Slope (b)	0.0129	0.0047
Intercept (a)	-0.0009	0.0104
Correlation coefficient (γ)	0.9997	0.9981
Standard Deviation (SD)	9.9827 x 10^3	0.0163

V. Conclusions

Atomoxetine hydrochloride reacts with gold(III) to form stable violet coloured 1 : 1 complex at pH 4.0. Spectrophotometric and derivative spectrophotometric methods are developed based on this reaction. They are sensitive for the assay of both atomoxetine hydrochloride and gold(III). The tolerance limit of the excipients and the foreign ions in derivative methods is found to be generally 10 – 20% greater than that of the zero order method. The present spectrophotometric methods are direct, simple and highly selective for the determination of gold(III) or atomoxetine hydrochloride. Further, the methods can easily be employed by ordinary clinical laboratories as the methods can be carried out using a simple colorimeter.

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Unani Description of Sumaq (*Rhus Coriaria Linn.*) and its Scientific Report

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Abstract- Plants have played a vital role in the prevention and treatment of diseases since prehistoric times. WHO estimates that 65%-80% of the world's population use traditional medicines, as their primary form of health care and most of the diseases have been treated by administration of plant or plant products. Sumaq (Rhus coriaria Linn.) is most useful herbal medicinal plant in India its post of fruits possess medicinal property. During the last few years the phytochemistry of the Sumaq is been achieved regarding the biological activity and its medicinal applications. It is now considered as a natural product for development of medicines against various diseases and also for the development of industrial products. This review gives a keen view mainly on the biological activities of the Sumaq and some of their compounds isolated, pharmacological actions of the Sumaq extracts and plausible medicinal applications of Sumaq along with their safety evaluation.

Keywords: *rhus coriaria, sumaq, unani medicine.*

GJMR-B Classification : *NLMC Code: WB 55*

Strictly as per the compliance and regulations of:

Unani Description of Sumaq (*Rhus Coriaria Linn.*) and its Scientific Report

Haqeeq Ahmad [α], Faiyaz Ahmad [σ], Izharul Hasan [ρ] & Shabbir Ahmad [ω]

Abstract- Plants have played a vital role in the prevention and treatment of diseases since prehistoric times. WHO estimates that 65%-80% of the world's population use traditional medicines, as their primary form of health care and most of the diseases have been treated by administration of plant or plant products. Sumaq (Rhus coriaria Linn.) is most useful herbal medicinal plant in India its post of fruits possess medicinal property. During the last few years the phytochemistry of the Sumaq is been achieved regarding the biological activity and its medicinal applications. It is now considered as a natural product for development of medicines against various diseases and also for the development of industrial products. This review gives a keen view mainly on the biological activities of the Sumaq and some of their compounds isolated, pharmacological actions of the Sumaq extracts and plausible medicinal applications of Sumaq along with their safety evaluation.

Keywords: *rhus coriaria*, sumaq, unani medicine.

I. Introduction and History

Rhus coriaria Linn .is commonly known as Sumaq and the leaves have long been well known in Europe and in the East.. It belongs to the family Anacardiaceae[1]. Theophrastus and Dioscorides described it as the fruit of plant used for tanning. Abu Hanifeh in his "Book of plants" says that Sumaq has bunches of small, intensely red berries, and it does not grow in part of the land of the Arabs except Syria[2,3]. The fruit rind of Sumaq is commonly known as Post Sumaq which is medicinally used and has astringent property.[4]

a) *Taxonomical Classification*

Kingdom: Plantae, Sub kingdom: Tracheobionta, Super division: Spermatophyta, Division: Magnoliophyta, Subclass: Rosidae, Order: *Sapindales* , Family: *Anacardiaceae*, Genus: Rhus, Species: *Rhus coriaria* Bionomial name: *Rhus coriaria* Linn.

b) *Vernacular Names*

Sumaq (fruit of Rus coriaria Linn) is known by different names worldwide including Indian sub continent as follows: Persian: Samaka, Samak, Sumaq, Hindi: Tatrak, Tatri, Arabic: Timtima, Tamtam, Sumak, Urdu: Sumaq, English: Sumach, Sumak, Sanskrit: Tandidik, Bengali: Sumok, Kashmiri: Samak, Chokmusur, Marathi : Sumak, Punjabi: Minas, Ninawa, Samakdana, Tungla.[5,]

II. Habitat and Distribution

The plant is globally distributed in temperate and tropical regions and can grow on marginal lands. The plants have shallow spreading root system that prevent soil erosion and can grow on poor eroded soil. Most common sumac grown commercially on global scale is *R. coriaria* in Mediterranean and Middle East, having been cultivated for several centuries to produce a material of high quality for tanning. It is found growing naturally in region of Mediterranean, South east and central and northern regions of Turkey [6,7,8]

a) *Botanical Description*

R. coriaria is a 1-3 meter heigh shrub or small tree. The leaves are imparipinnate with 9-15 leaflets. The inflorescence is a compact and erect panicle, the flowers are small and greenish white. The fruits are a small flattened drupe the size of the lentil of red colour, containing one lenticular polished brown seed [3,8]

b) *Description of Sumaq As Reported In Unani Literature*

Sumaq is a fruit of a plant of *Rhus coriaria*. This plant grows on hard soil its height is up to 2 meter. Leaves are large and reddish in colour. Fruits aggregate equal to the size of the Mako (*Solanum nigrum*). The peel of the fruit is bitter in taste. The bitterness is increased when fruit ripes properly. There are two varieties of Sumaq

1. Sumaq Bustani (Garden Sumach)
2. Sumaq Kohi (Mountain Sumach),

Mountain Sumach has more dryness than that of Garden Sumach [9].

Part Used Medicinally Fruit, fruit rind, extract and peel is used in Italy[10].

Mizaj (Temperament)
Cold and Dry in 2 degree [11,12]

Pharmacological actions

Qabiz (astringent) , Habisuddam (styptic), Maqawwie Medah (stomachic) , Hazim (digestive) ,

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Musakkin (sedative), Rade (repellent), Mushtahi (appetizer) and Dafe Taffun (antiseptic).[13,14,15]

Mawaqe Istamal (Therapeutic uses)[11,12,16.]

1. It is used to increase the protection property of mucosal layer of stomach, it protects stomach and intestinal irritation due to bile.
2. It is used in the treatment of nausea and safrawi qai wa safrawi Diarrhoea.
3. Sumaq is used to prevent haemoptysis
4. Due to astringent property Sumaq is used to strength the mucous membrane of stomach as well as intestine
5. Sumaq is also used in haemorrhage in any part of the body due to presence of tannins
6. The sanoon of Sumaq is also used in stomatitis and Pyorrhoea
7. The Joshanda of Sumaq is used for black and shining hairs
8. Sumaq is also used in dysentery
9. The massage of Sumaq is very useful in warts of piles
10. The fine powder of post Sumaq is mixed with alcohol and used in leucorrhoea and menorrhagea
11. In conjunctivitis its Qutoor is very useful.

Mazarrat (Toxicity)[17]
For chest and liver (For cold temperament person)

Musleh (Correctives) [9]
Mastagi (Pistacia lentiscus), Anisoon (Pimpinella anisun) and Badiyan (Foeniculum vulgare Mill.)

Badal (Substitutes)
Sirka (Vinegar), Zarishk (*Berberis vulgaris*) [9]

Miqdare khurak (Dose)[11, 16]
4-5gm
3-5gm

Murakkabat (Compound formulations)
1. Hubb-e- Sumaq [18]
2. Qurs-e –Ziabaetus[19]
3. Jawarish Tabasheer [19]
4. Annushdaro Sada[17]
5. Jawarish Zarishk [19]
6. Sufuf Shahatrah [19]

c) Description of Sumaq (Rhus coriaria Linn.) as given in Modern Literature

The modern description of Sumaq can be studied under the following categories:

III. Geographical Distribution

Sumaq (Rhus coriaria Linn.) belongs to the family Anacardiaceae. This family has 60 genera and some 600 species; mainly tropical shrubs and trees. Rhus includes 250 species. Sumaq leaves used in dyeing, tanning and fruits as a medicine The leaves have long been well known in Europe and in the East. Theophrastus and Dioscorides described it as the fruit of plant used for tanning. Abu Hanifeh in his "Book of plants" says that Sumaq has bunches of small, intensely red berries, and it does not grow in part of the land of the Arabs except Syria[2, 3] . The fruit rind of Sumaq is commonly known as Post Sumaq which is medicinally used and has astringent property.

a) Macroscopic Features

Fruit: Small dark brown, hairy , hard, laterally compressed drupe; 3.5to 4.0 cm in length and 2 to 2.5 cm in width; persistent calyx.[20]

Seed: Small, 0.3 to 0.5 cm in length and 0.2 to o.3 cm in width; brown polished and hard, odour spicy.[20]

b) Microscopic Features

Fruit: Transverse section shows cuticle and a single layered epidermis with characteristic horn shaped multicellular trichomes , mesocarp 5or 6 layered cells are thin walled, parenchymatous, filled with oil bodies and tannin , endocarp tissue crushed.
The fragments of the epidermal fruit wall cells in surface view are polygonal and moderately thick walled ; show the presence of abundant , small circular cicatrices with the epidermal cells radiating around it.[20]

Seed: Transverse section of mature seed shows testa differentiated into a radially much elongated thick walled outer layer of palisade cells filled with some brownish contents; followed by a layer of elongated but much smaller radial cells with lignified walls ; the inner integumentary cells are also composed of radially much elongated thick walled palisade cells, similar to outer layer ; endosperm tissue with numerous oil globules followed by tissues of the embryo present. The fragments of the dark brown testa in surface view show unifomily thick –walled, almost square or rectangular cells.[20]

Powder: Powder is dark brown , bitter in taste; shows characteristic in horn-shaped multicellular trichomes, large and small palisade cells from testa, fragments of fruits walls with cicatrices; testa of the seeds; embryo and oil globule. [20]

IV. Scientific Studies

a) Phytochemical studies

Phytochemicals in *R. coriaria* are being used as antibacterial, antidiarrheic, antidysenteric, antihepatoxic, antiseptic, antispasmodic, antiviral, astringent, candidicide, hepatoprotective, hepatotonic, protisticide, analgesic, antigastric, anti-inflammatory, antioxidant, antiulcer, fungicide, cyclooxygenase-inhibitor and lipoxygenase inhibitor due to their contents of ellagic acid, gallic acid, isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid .[21]

V. Pharmacological Studies

a) Antibacterial activity

The hydro alcoholic extracts of *Rhus coriaria* ripe berries were studied against five clinical bacterial strains (Methicillin-resistant *Staphylococcus aureus* (MRSA), multi-drug resistant *Pseudomonas aeruginosa*, *enterohhemorrhagic Escherichia coli* O157 (EHEC), *Proteus vulgaris* and *Klebsiella pneumonia*). *Bacillus subtilis* ATCC6633 was used as a reference strain. The zone of inhibition varies depending on bacterial species and type of extract. The results showed that the antibacterial activity of *R. coriaria* was more effective against Gram-positive bacteria than Gram-negative [21] .

b) Anti diabetic and antioxidant activity

Single dose administration of the extract significantly reduces postprandial blood glucose by 24% (at 5 hrs). In the long term experiment, on the day of 21, postprandial blood glucose (PBG) was found to be significantly lower (by 26%) compared to diabetic control group. The plant extract raised markedly serum high-density lipoprotein (HDL) by 34% and also reduced low-density lipoprotein (HDL) by 32%. Also it had noticeable antioxidant effects by elevating superoxide dismutase (SOD) and catalase(CAT) activities by 46% and 77%, respectively. However it did not show a strong effect on glutathione peroxidase (GPX) activity. The extract inhibited maltase and sucrase activities by 44% and 27%, respectively. However it made no changes in the transcript levels of INS and GLUT-4 genes. It can be concluded that constituents of *Rhus coriaria* fruits have effective components which can be utilized as useful herb for alleviation of diabetes complications 22,.

c) Anti hyperlipidemic activity

In an experiment, one-day-old broiler chickens (Ross 308) were used to investigate the effects of sumac fruit (*Rhus coriaria* L.) powder (SFP) on plasma concentrations of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-c), low density lipoprotein (LDL-c), very low density lipoprotein (VLDL-c) and plasma fasting blood sugar (FBS), as well as proportional abdominal fat.. The birds were fed the basal diet (Z-SFP) or diets supplemented with 2.5 g SFP (L-SFP), 5 g SFP (M-SFP) and 10 g SFP (H-SFP) per kg diet. During the whole experimental period the H-SFP birds had a higher feed intake than the Z-SFP and L-SFP birds, though the H-SFP birds had higher feed conversion ratio compared with birds in the other treatments. No significant differences for body weight gain were recorded between the treatments. The M-SFP and H-SFP birds had lower plasma.

TC and VLDL-c concentrations than the Z-SFP and L-SFP birds. No significant differences between the treatments were indicated for plasma TG, HDL-c and LDL-c concentrations. Moreover the plasma FBS concentration of the H-SFP birds was lower than the birds in treatments Z-SFP and L-SFP, but no significant differences were observed between the other treatments[23] .

d) Antifungal activity

Phytochemical investigation of the ethanolic extracts of the seeds *Rhus coriaria* Linn. (Anacardiaceae) afforded three new aromatic compounds identified as 1-methoxy-4-hydroxy- methylene naphthalene (coriarianaphthyl ether), 7-methoxy-5-methyl benzene-4-al-oic acid(coariariaoic acid) and 1-dodecanoxy-2,8-dihydroxy-anthracene-15oic acid (coriarianthracenylester) along with known phytoconstituents n-tetracosane, n-pentacosane, anise alcohol, p-hydroxy benzyl alcohol, methyl lawsone and 2- hydroxyl methylene naphthaquinone. The structures of all the isolated compounds have been identified on the basis of spectral data analysis and chemical reactions. All the new compounds showed the antifungal activity[24] .

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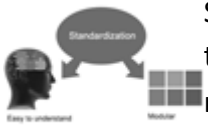

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3. Submission of Manuscripts,

4. Manuscript's Category,

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12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Administration Rules Listed Before Submitting Your Research Paper to Global Journals Inc. (US)

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

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