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of Medical Research: B

Pharma, Drug Discovery, Toxicology and Medicine

Drug-Induced Disparities

Triggered Nuclear Misreading

Highlights

Sepsis Macrovascular Disease

Frankincense (Boswellia Species)

Discovering Thoughts, Inventing Future

VOLUME 15 ISSUE 3 VERSION 1.0

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Rhodiola Rosea from the Selection of Traditional Applications to the Novel Phytotherapy for the Prevention and Treatment of Serious Diseases

By Dr. Rafie Hamidpour, Dr. Soheila Hamidpour, Dr. Mohsen Hamidpour, Mrs. Mina Shahlari, Mrs. Mahnaz Sohraby, Ms. Nooshin Shahlari & Ms. Roxanna Hamidpour

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Abstract- *Rhodiola rosea* is a remarkable herbthat has been a part of traditional medicine systems in order to stimulatethe nervous system, toprotect thebody against oxidative stress, free radical damage, inflammation, and virus infection. *Rhodiola rosea* is included among a class ofplant derivatives called adaptogen,an agent thathelps the body adapt to various stressors. Adaptogens have been claimed to treata wide variety of medical conditions, fromfatigue to cancer.

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Keywords: *antifatigue, antidepressant, alzheimer's disease, cancer and memory enhancement.*

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Dr. Rafie Hamidpour [α], Dr. Soheila Hamidpour [σ], Dr. Mohsen Hamidpour [ρ], Mrs. Mina Shahlari [ω], Mrs. Mahnaz Sohraby [¥], Ms. Nooshin Shahlari [§] & Ms. Roxanna Hamidpour [χ]

Abstract- ***Rhodiola rosea*** is a remarkable herb that has been a part of traditional medicine systems in order to stimulate the nervous system, to protect the body against oxidative stress, free radical damage, inflammation, and virus infection. ***Rhodiola rosea*** is included among a class of plant derivatives called adaptogen, an agent that helps the body adapt to various stressors. Adaptogens have been claimed to treat a wide variety of medical conditions, from fatigue to cancer.

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This research paper, suggests that ***Rhodiola rosea***, in addition to cure common disorders such as depression, binge eating, anorexia, generalized anxiety disorders, and physical and mental fatigue, might contribute to prevent, reduce and treat serious diseases such as Alzheimer's disease, Parkinson's disease, cardiovascular disease, diabetes, and cancer. The aim of our future research is to extract ***Rhodiola rosea*** into the filtration equipment and then, with purification and extended quality control, produce tablets for the animal trails.

Keywords: antifatigue, antidepressant, alzheimer's disease, cancer and memory enhancement.

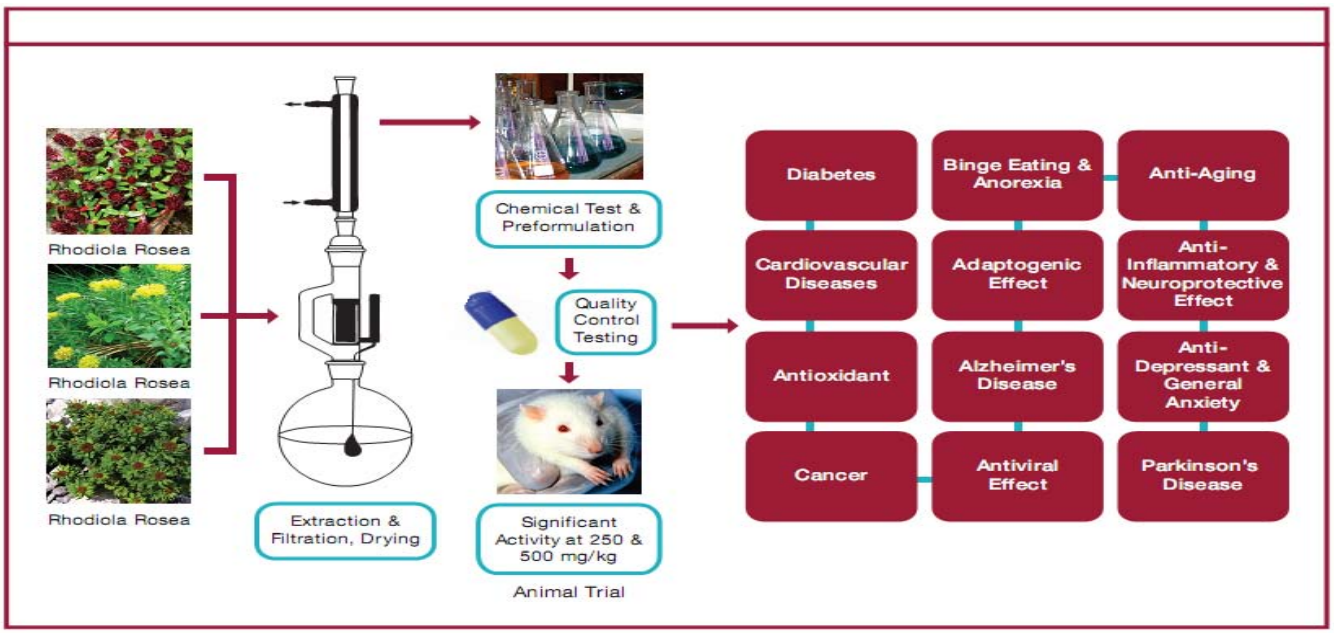

Figure 1

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I. Introduction

Rhodiola rosea, also known as golden root or *Lignum rhodium,* is a perennial herbaceous plant in the Crassulaceae family which has been used as a natural medicine from ancient times. This perennial plant reaches a height of 30-70 cm with a thick rhizome and yellow, fragrant flowers. It is a remarkable herb that is valued in traditional medicine in Eastern and Northern Europe, Asia, China, and Russia for its unique pharmacological activity.[1] The plant has been categorized as an "adaptogen" by Russian researchers due to its ability to elevate body resistance to physical, chemical or biological stressors, treat fatigue, promote longevity, and support cognition and mood wellbeing.[2] *Rhodiola rosea* (SHR-5 extract) has been indicated as an adaptogen in the situation of fatigue, poor mental performance and depression.[3] *Rhodiola rosea* phytochemical extracts, are the source of important biological activities which is used widely in the treatment of a wide range of diseases like those of the nervous and cardiovascular systems, Alzheimer's and Parkinson's disease, cancer, and inflammatory diseases.[4] The studies of pharmacological activities of *R. rosea* have revealed its hepato-protective and Monoamine oxidase A (MAO-A) inhibitory effects, in addition to the antiviral and antibacterial activities of this plant.[5]

Phenylethanoid (salidroside, ρ-tyrosol), phenylpropanoid glycoside (rosarin, rosavin, rosin) and monoterpene (rosiridin) are responsible for the bioactivity of *R. rosea*. Salidroside, rosarin, rosavin, rosin, and ρ-tyrosol are the most critical plant constituents used for therapeutic activities. Salidroside and ρ-tyrosol have been found in all *Rhodiola* species but the other active glycosides: rosavin, rosin, and rosarin have not been detected in other genus of *Rhodiola* species. The compound rosavins (rosavin, rosin, and rosarin) are the compounds that contain the highest percent of *R. rosea*, which was not identified in other species. The compound salidroside is the most biologically active compound which shares many of its effects with rosavin.[5,6] The absence of adverse drug interactions and side effects associated with *R. rosea* in the clinical trials make it possible to be used as a safe medication. Rhodiola rosea also can be applied as an adjuvant to enhance therapeutic effects of other medicines in a number of disorders such as chronic pneumonia, chronic tuberculosis, vascular dystonia, cancer (reduction of metastasis), and in reducing the debilitating effects of radiotherapy and chemotherapy.[3,5]

II. Common Names

Rhodiola rosea has numerous common names. Some of the best known names include Arctic root, Golden root, King's crown, Lignum rhodium, Orpin rose, Rose root, Sedum rhodiola, and SHR-5 extract. The term "arctic root" is used as a general name, however; arctic root is actually a trademark name for the specific commercial extract.

III. Chemical Composition

The phytochemical analysis of the *Rhodiola* species has shown that the major beneficial components include salidroside and tyrosol, which are rich in the rhizomes.[7] The dried rhizomes contained 0.05% essential oil. Terpenes and volatile compounds have been isolated from *Rhodiola rosea*. As shown in Table 1, Myrtenol (36.9%), trans-pinocarveol (16.1%), geraniol (12.7%), Cumin alcohol (12.1%), Linalool (2.7%), Perilla alcohol (1.7%) and dihydrocumin alcohols (12.1%) are the most abundant volatiles detected in the oil.[8] Geraniol and Myrtenol are responsible for the rose like odor of the plant. A total number of 86 chemical compounds were identified in *R. rosea* roots. The principal components are phenylpropanoids (rosavin, rosin, and rosarin), Phenylethanoids (salidroside, ρ-tyrosol) and a monoterpene (rosiridin) which are responsible for the pharmacological effects of *R. rosea.*[8,3] Rosiridin has attracted particular interest because of its effect in depression and senile dementia. Rhodioloside and salidroside active principles of the SHR-5 extract were found to have neuro-, cardio- and hepato- protective activities and can be effective in the prevention of a number of disorders related to neuro-endocrine and immune system. Three rosavin compounds (rosavin, rosin, and rosarin) which are unique to *R. rosea* (the most used species of Rhodiola genus) might be responsible for antidepressant, anticancer, neurotropic, and hepato- protective effects of this herb.[3]

Table 1. Chemical Composition of oil of Rhodiola rosea

Compound	Percentage
Linalool	2.7
Octanol	13.6
6,6-dimethyl-bicyclo[3,1,1] hept-2-ene-2-carboxaldehyde	1.0
Trans-pinocarveol	16.1
Myrtenol	36.9
Geraniol	12.7
Myrtanol	1.0
Perilla alcohol	1.7
Dihydrocumin alcohol	2.1
Cumin alcohol	12.1

Ref: [8] J Essen Oil Res 2005; 17(6):628-9.

a) Antioxidative effect

The imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanism causes oxidative damage to the proteins, membrane lipids and nucleic acids in the cells. The increased generation of ROS damages the mitochondria, the power house of the body, which account for reducing the ability of maintaining energy at the cellular level and results in muscular atrophy and

muscle fatigue, leading to the decreased performance of an individual.[9]

Antioxidants are natural substances that prevent or delay some type of cell damages and protect the body against the oxidative stress and free radicals. Various *Rhodiola* species have shown significant antioxidant activities. Among the 28 different compounds identified in *R. rosea*, P-tyrosol, salidroside, and five salidroside-like glycoside (Rhodiolin, rosiridin, rosarin, rosavin, and rosin), possess strong antioxidant activities.[10]

Polyphenols in *R. rosea* neutralize oxidative reactions, which are induced by free radicals since they are excellent donors of protons and electrons. In addition, polyphenols, due to thier metal chelating properties, are able to decrease oxidative stresses, induced by transition metals.[12]

Salidroside (SDS), a major component extracted from *Rhodiola rosea*, is a glucoside of tyrosol which possess a broad spectrum of pharmacological properties including strong antioxidant activity. Salidroside induces its antioxidant effects to the cells by preventing collection of intracellular ROS, restoring the impaired mitochondria function and mitigating oxidative-stress-induced apoptosis.[11]

Production and detoxification of Reactive Oxygen Species (ROS) are of major importance in regulation of erythropoiesis (formation of red blood cells). Salidroside plays an essential role in maintaining normal erythropoiesis through the up-regulation of antioxidant defense mechanism. Salidroside could mediate its effect as blood tonic supplement and adaptogen. Patients with anemia and malhypoxia can take advantage of SDS as an adjuvant for erythropoietin (EPO) or other erythropoiesis-stimulating agents. This compound also defends erythroblasts against oxidative stress through up-regulating the expression of antioxidant molecules, glutathione peroxidase, and thioredoxin, and it also nullifies ischemia-induced cardiomyocyte death through suppressing ROS overgeneration.[11,13]

b) Effect on cancer cells

Cancer is a class of diseases characterized by out-of-control cell growth. Complete eradication of cancer without damage to the rest of the body is the goal of the treatment. Some plant extracts that indicate potential as an anticancer agent have shown to be useful for the treatment or prevention of the cancer with minimal toxicity, and they act synergistically with cytostatic to reduce their toxicity. The study showed that the use of *R. rosea* extract in combination with the antitumor agent cyclophosphamide increased the anti-tumor and antimetastatic efficacy of the drug.[14]

The results of investigation *in vivo* show that *R. rosea* extract has cytotoxic effect on tumor cell line through its major component, polyphenols. The cytotoxicity effect of polyphenols on tumor cells are induced by reaction oxygen species (ROS) mediated mechanisms. Polyphenols including tannins and gallic acids, induce apoptosis in tumor cells by increasing intracellular peroxides. [15,16] The results show that salidroside, a component isolated from plants that belong to the *Rhodiola* genus, causes growth inhibition in several human cancer cell line in concentration between 1μg/ml and 32μg/ml dose dependently by induction of G1-phase and/or G2-phase arrest. A number of studies have investigated the inhibitory effect of salidroside on the growth of stomach adenocarcinoma cells, leukemia cells, and parotid carcinoma cells *in vitro*. In a few studies performed in China, was found that Salidroside could inhibit tumor-induced angiogenesis in mice.[17]

Breast cancer is the most common cancer diagnosed in women in the United States. It develops by the mammary cell proliferation induced by estrogen. Resistance of estrogen receptor negative (ER^-) tumors to anti-hormone therapy is the main concern in breast cancer treatment. Investigations of the effects of salidroside on the breast cancer showed its inhibitory properties on human breast cancer MDA-MB-231 cells. The result indicated that salidroside in concentration between 5μm and 80μm dose dependently induced cell-cycle arrest and apoptosis cell death in ER-negative and ER-positive tumors in human breast cancer.[18]

Thyroid cancer is the most frequent endocrine neoplasia and accounts for about 2% of cancer-related deaths. Management options for thyroid cancer include total or near total thyroidectomy, radioiodine therapy and pharmacotherapy. These patients may have neuropsychological concerns such as depressive moods or developed cardiovascular problems such as hypertension, electrocardiogram abnormalities, and diastolic dysfunction. In numerous studies, *R. rosea* has demonstrated CNS stimulating, neuro-, cardio-protective and antidepressant effects. Since most of these symptoms are in fact the clinical aspect of hypothyroidism, *Rhodiola rosea* is recognized to aid in patient preparation during the hormone withdrawal period. Oxidative stress increases when thyroid hormones are missing during hypothyroidism. Studies in rats reveal that supplementation with *R. rosea* extract can protect cells from oxidative injuries in dose-dependent manner. This finding has also been replicated in human. *Rhodiola rosea* have potentially additional benefits as an adaptogen that tends to be a regulator, having normalizing effects on the organism. Hypothyroidism can be considered as a stressor and then *R. rosea* as an adaptogen that could help the organism's responding.[19]

c) Alzheimer's Disease

Alzheimer's disease (AD) is a progressive brain disorder characterized by the memory and

cognitive impairments. Neuropathologically, AD is defined by the accumulation of amyloid plaques and neurofibrillary tangles in certain region of the brain which are important in memory and can cause the loss of synaptic connection between cells. One of the most important parts of unraveling the AD mystery is discovering what causes the disease. It has been suggested that oxidative stress and dysfunction of neurogenesis play important roles in pathogenesis of AD.[20] Beta-amyloid (Aβ) peptide, the hallmark of Alzheimer disease induces an oxidative damage to neurons and finally causes neurons death. Reduced levels of anti-oxidative activity have been observed in the specific regions of the central nervous system of AD patients.

Now researchers are paying great efforts to find potent natural antioxidant with neuroprotective potentials. Salidroside, an active compound occurring naturally in *Rhodiola rosea* L. is protective against (Aβ)-induced oxidative stress by the induction of antioxidant enzymes, thioredoxin (Trx), heme oxygenase-1 (HO-1), and peroxiredoxin- 1(Prxl); the down regulation of pro-apoptotic protein Bax and the up regulation of anti-apoptotic BcL-X1. Pathophysiology of neurodegerative diseases such as AD has shown that Aβ is associated with ROS generation which leads to mitochondrial dysfunction, lipid peroxidation and apoptosis. Exposure to ROS also inhibits neurogenesis, which is the onset of cognitive impairments and memory deficits. Salidroside could decrease the intracellular ROS level and restore the abnormal mitochondrial membrane potential (MMP). The neuroprotective effect of Salidroside may offer long-term protection in the pathogenesis of AD.[20,21]

d) Adaptogenic and antifatigue effects

Adaptogens are unique group of herbal ingredients which help strengthen the body's response to stress, enhance its ability to cope with anxiety, and fight fatigue. They have the unique ability to adapt their function according to the body's specific needs and do not disturb bodily functions at normal levels. *Rhodiola rosea* is known as a plant's adaptogens because it possesses anti-fatigue and anti-stress activities that can increase mental and physical working performance against a background of fatigue or stress.[22] The phenylpropanoid glycoside called salidroside, flavonoids, Phenolic, polyphenolic, and flavolignas are thought to be the main components of stress- protective and adaptogens of *Rhodiola rosea*. Other constituents isolated from R. rosea, including rhodioniside, rhodioloside A-E, rhodiolin, rosin, rosavin, rosarin, rosiridin, rosiridol, rhodalgin, acetylrhodalgin, and lotaustralin, might also be responsible for R. rosea's stimulant or adaptogenic effects. Such compounds can play an active role in increasing energy, stamina, strength and mental capacity required in fight to fight situation to help the body to adapt and resist physical, chemical, and environmental stresses.[22,23]

Clinical efficacy of adaptogens in behavioral and mental disorder has been reviewed. It is now accepted that adaptogens have shown anti-fatigue, anti-depressant, anxiolytic, nootropic, and CNS stimulating effects. Adaptogens do not possess any side effects of conventional drugs such as addiction, tolerance and abuse potentials, or impair mental function, neither do they cause psychotic symptoms with long term use.[24]

Neuro-degenerative disorders characterized by the progressive loss of structure or function of neurons in the brain region involved in learning and memory. *Rhodiola rosea* as an adaptogen could induce a positive effect in neuro-degenerative disorders due to their inhibitory effects on the formation of p-SAPK (phosphorylated stress-activated protein kinase). Related data may be considered to add further support to the hypothesis that adaptogens have beneficial effect on mental performance and cognitive function.[22] The key point of action of adaptogens on stress appears to be related to the regulation of homeostasis via hypothalamic-pituitary-adrenal axis and regulation of molecular chaperones, stress-activated c-Jun, N-terminal protein kinase, forkhead box O transcription factor DAF-16, cortisol, nitric oxide (NO) and beta-endorphin.[24] The optimal corticosteroid level is required for efficient cognitive function. Significant changes (up or down) in circulating levels of corticosteroids have been accepted as the reason for cognitive impairment. Regulatory effects of *R. rosea* on the basal level of salivary cortisol results in an improvement in cognitive function.[3]

Rhodiola rosea combines well with other adaptogens and tonics in appropriate dosages. The herbal drug ADAPT-232 is based on the synergistic effect of the three most efficient adaptogen plants, *Rhodiola rosea*, *Schisandra Chinensis* and *Eleutherococcus senticosus* in a fix combination. Administration of single and repeated doses of ADAPT-232 has been shown to increase physical energy as well as mental performance and cognitive function.[25] ADAPT-232 significantly increases secretion and release of stress hormones, neuropeptide Y (NPY) and Heat Shock Protein 72 (Hsp 72) which increase tolerance and adaptation to stress. These pathways contribute to the anti fatigue effect of ADPAT, increase the attention and improve the cognitive function.[24]

Furthermore, a number of studies have investigated the effects of ADAP-232 on pneumonia patients. Clearly, adjuvant therapy on pneumonia patients with ADAPT-232 has a positive effect on the recovery of the patients, by decreasing the duration of the acute phase of the illness, increasing mental

performance of the patients during the rehabilitation period and by improving their quality of life.[25]

e) Anti-depressant and general anxiety

Depression is a severe despondency and sadness accompanied by a feeling of desperation and inadequacy. The mechanism of depression is complex. The therapeutic effects of anti-depressants such as Tricyclic antidepressants (TCAs), Monoamine oxidase inhibitors (MAOLs) and Selective serotonin reuptake inhibitors (SSRIs) come with a number of side effects like psychomotor impairment and dependence liability.[26]The use of Alternative Medicine especially natural products for the treatment of mental disorders have been increased in the U.S and worldwide. The most common reason for people to use complementary therapies is that they want to avoid the common side-effects of prescription anti-depressant drugs. A few natural psychotropics have been more extensively examined in well-designed, placebo-controlled, double-blind studies. *Rhodiola rosea* is one of these second-tier natural products for mood disorders.[27] The standardized extract SHR-5 (3%rosavin and 0.8% salidroside) from *R. rosea* have a significant antidepressant activity in mild to moderate depression. The symptoms evaluated were emotional instability, decreased motivation, cognitive complains and susceptibility to stress.[28] Significant improvement in the overall symptom of depression and mood deficiencies was observed in a 6-week monitoring study in Sweden, which *R. rosea* was given daily with a dosage of two tablets a day, each containing 170mg of the extract.[28] The role of serotonin, a monoamine neurotransmitter, is usually known and associated with depression, however, serotonin also has some cognitive functions, including the enhancement of memory and learning. Regulation of serotonin at synapses is a major mechanism of action possibly contributing to pharmacological antidepressants. Central and peripheral serotonin levels decreases in patients with depression. Monoamine oxidase type A has an important role in degradation of biogenic amines such as epinephrine, norepinephrine, and serotonin. Monoamine oxidase inhibitors (MAOIs) prevent the breakdown of monoamine neurotransmitters including serotonin and therefore increases the concentrations of neurotransmitter in the brain. MAOIs therapy with synthetics drugs are known to interact negatively with other medications and even with food. MAOIs can cause death if they are taken in overdose extent. There is evidence that R. rosea acts as monoamine oxidase inhibitors and influences the level and activity of biogenic monoamines such as serotonin, norepinephrine, and dopamine in the nerve terminal. *Rhodiola rosea* inhibits the activity of the enzymes responsible for monoamine degradation (monoamine oxidase and catechol-0 methyl transferase).[4,3] General anxiety disorder (GAD) is a common disorder that involves chronic worrying, nervousness and tension. There are different types of medication for GAD, including antidepressants, Benzodiazepines, and serotonin reuptake inhibitors. Patients who do respond to conventional treatment often experience adverse side effects that may interfere with their consistency. *Rhodiola rosea* is safe and tolerable alternative medicine. Administration of R. rosea in dosages of 2-3 capsules each containing 100-170 milligrams daily approximates to the perfect dose to gain beneficial effects.[29]

f) Anti-inflammatory and neuroprotective effect

In general, inflammation is a localized reaction of the body tissues to infections, irritation, injuries, or disorders of the immune system which produce redness, warmth, swelling, and pain. As we age, the level of inflammatory immune cytokines increases and we get vulnerable to a number of inflammation-linked diseases, such as cancer, arthritis, muscle weakness, fatigue, sleep disorder, Alzheimer's and Parkinson's disease. An enormous amount of research has demonstrated the link between chronic low-level brain inflammation and elevated brain glutamate levels, which are a neurotransmitter normally involved in learning and memory. In some cases, glutamate can be an excitotoxin that is involved in nerve-cell death in various neurodegenerative disorders including Alzheimer's and Lou Gehrig's disease. Glutamate not only influence amyloid β production (the cause of Alzheimer's disease), but also amyloid β can change the levels of glutamate in the brain which increase the vulnerability of cortical neurons to glutamate cytotoxicity. It has been shown in several studies that *R. rosea* could improve inflammation and neurotoxicity in cortical neuronal cells. *Rhodiola rosea* modulates the neuronal over action and endogenous anti-inflammatory.[30]

Microglia, a type of glial cell, acts as the first and main form of active immune defense in the central nervous system (CNS), and thus plays a key role in the inflammatory reaction. Inflammatory process, in the central nervous system leads to neuronal cell death, and inflammatory response is mediated by the activated microglia, which remove the damaged cell by phagocytosis. The chronic activation of microglia may in turn cause neuronal damage through the secretion of cytotoxic molecules such as proinflammatory cytokines (interleukin-1ß (IL-1), IL-6 and TNF-a), proteases, and reactive oxygen species (ROS), and nitric oxide (NO). Therefore, suppression of microglia-mediated inflammation can appear to be the most promising option in neurodegenerative disease therapy. Since overproduction of NO plays an important role in neuroinflammatory disease, the effect of the *R. rosea* on nitric oxide production was investigated in lipopolysaccharide (LPS)-induced microglia cells.

Rhodiola rosea has shown to strongly inhibit NO production and the expression of Inducible nitric oxide synthase (iNOS), the key enzyme for NO in LPS-stimulated microglia cells.[30]

g) Antiviral activity

The influenza is an acute infectious disease caused by an RNA virus of the family orthomyxovirus. Influenza virus infects the epithelial cells of respiratory tract that causes acute pulmonary diseases. Influenza outbreak usually occurs in winter, killing numerous people in pandemic years. The epidemic outbreaks of influenza are associated with influenza virus type A and B. Type C virus is associated with minor symptoms. Two neuraminidase inhibitors have been approved by FDA (zanamivir and oseltamivir) to treat influenza virus infection. Both of these inhibitors are active against influenza virus A and B, however, they have several toxic effects in the digestive and autonomic nervous system. The flavonols Kaempferol, Herbacetin, Rhodiolinin, Rhodionon and Rhodiosin were isolated from Rhodiola rosea. The compounds showed neuraminidase inhibitory and anti-influenza virus activities. The *in vitro* anti-influenza virus activities of flavonoids were evaluated using two influenza viral strains, H1N1 and H9N2, testing their ability to reduce virus-induced cytopathic effect (CPE) in MDCK,Madin-Darby Canine Kidney Cells (virus tissue culture). Anti-influenza activity depends on the position and the number of hydroxyl groups on the flavonoids backbone. Kaempferol showed the highest activity against two influenza viruses, H1N1 and H9N2 with the half maximal effective concentration (EC50) values of 30.2 and 18.5μM.[31]

Coxsackievirus B3 (CVB3) is important human pathogen that belongs to picornavirus family. CVB3 is the most common cause of viral myocarditis, a serious disease that can further lead to dilated cardiomyopathy and cardiac failure and also often induce pancreatitis and aseptic meningitis. Although a few vaccines have been reported to be effective in a murine CVB3-induced myocarditis model, there are no effective therapeutic agents against CVB3 for the clinic up to now. Slidroside (p- hydroxyphenethyl-β-D-glucoside) which is extracted from *R. rosea* demonstrated antiviral activity while not affecting the normal physiological function of the host cells. Salidroside exhibited obvious antiviral activity *in vitro* and protected myocardial cells against CVB3 infection. The antiviral activities of salidroside against CVB3 may be related to modulating serum superoxide dismutase (SOD), serum nitric oxide (NO), serum catalase (CAT), and serum Malondialdehyde (MDA) activities to protect heart muscle against the harmful effect of free radicals. Also salidroside has the ability to increase the hemoglobin capacity to carry oxygen, which provides protection for the myocardial cells from hypoxemia. Since salidroside also has shown antiviral activities against CVB3 *in vitro,* the findings have significant implications for a potential therapeutic agent for treatment of viral myocarditis and influenza virus infections which is worthy of further future researches.[32]

h) Antidiabetic

The anti-diabetic effects of dietary administration of Rhodiola-water extract on streptozotocin (STZ)-induce diabetes rat model were investigated. STZ is a toxin with the ability to damage pancreatic beta cells, resulting in hypoinsulinemia and hyperglycemia. The study used STZ mice as a model because it is considered an appropriate model to assess mechanisms of diabetes and evaluate potential therapies. Three days administration of Rhodiola-water extract in STZ-diabetic rats resulted in an increase of glucose transporter subtype 4(GLUT 4) in skeletal muscle and a reduction of phosphoenolpyruvate carboxykinase in liver. It has been reported that Rhodiola-water extract have a long-term blood glucose level control effect and improves hyperglycemia by an increase of beta-endorphin secretion from adrenal gland to activate opioid μ- receptors to achieve the higher of GLUT 4 gene expression in STZ rats model.[33]

Evidence in both experimental and clinical studies shows that increased oxidative stress is the common pathogenic factor causing diabetic mellitus and its complication. Diabetes is a chronic metabolic disorder characterized by hyperglycemia and the inability of tissues to utilize glucose. Hyperglycemia and fluctuation in blood glucose generate oxidative stress through overproduction of reactive oxygen species. Dietary *R. rosea* supplementation results in a significant reduction on blood glucose and lipid peroxide, increased levels of glutathione, glutathione peroxide, catalase, and superoxide dismutase (SOD) in the liver. *Rhodiola rosea* extracts may be effective for correcting hyperglycemia and preventing diabetic complications.[34] Managing diabetes without any side effect is still a challenge. Therefore, it is worth more investigation in the antidiabetic activity of natural products such as *R. rosea* on humans in the future.

i) Lifespan increasing effects

Recent studies on *Drosophila melanogaster* and *Caenorhabditis elegans* have shown that bioactive components of *R. rosea*, particularly salidroside and/or rosavins, may have an effect on lifespan and improve health spans. The plant adaptogens can induce their effects by different routes. Adaptogens can extend the lifespan by increasing an organism's resistance against the damaging effects of different stress conditions. The plants adaptogens such as *R. rosea* interfere with the localization of DAF-16, a forkhead/winged-helix transcription factor. The Caenorhabditis elegans DAF-16 transcription factor is

critical for diverse biological processes specifically longevity and stress resistance. *Rhodiola rosea* induce translocation of the DNF-16 transcription factor from the cytoplasm into the nucleus. DAF-16 in the nucleus reprograms the transcriptional activities favoring the transcription of a large number of genes involved in stress resistance and longevity. [35]

Moreover dietary conditions are another hypothesis for anti aging effect of *Rhodiola rosea*. The effect of *R. rosea* supplement on the lifespan of fruit fly depends on diet composition particularly on the protein-to-carbonate ratio. Dietary compositions with the protein-to-carbohydrate ratio less than 1 extends the lifespan by 15% to 21%, but diets with high protein-to-carbohydrate ratio or high caloricity do not support the beneficial action of *R. rosea* on longevity.[36] Hormesis is favorable biological responses to a low dose stress-induced stimulation resulting in biologically beneficial effects on growth, reproduction and longevity. Hormesis activates defense systems of the body and the defense process repair the damage caused by the toxin and also protect body against any additional stress. It can be hypothesized that the plants adaptogen like *R. rosea* act as a mild stressor leading to activate an adaptive response which protects the cells from stressful environments and increase the life span. In this way, it can be mentioned that adaptogen acts as hormetic agents. The findings of a study support the view that low doses of *R. rosea* extract (10-25μg/ml) works in a deliberate and systematic way in order to increase the stress resistance and lifespan of *C. elegans* between 10 and 20%, whereas the higher doses tested (250μg/ml) of *Rhodiola* showed a life span shortening of 15 to 25 percent. [35]

j) Cardioprotective effects

Hyperhomocysteinemia (high homocysteine level in the blood) is a major risk factor of cardiovascular disease. An abnormal accumulation of homocysteine, an amino acid that is produced by human body due to consuming meat, is related to various cardiovascular diseases such as coronary heart disease, stroke and peripheral vascular disease (fatty deposits in peripheral arteries). Homocysteine exert its adverse effect on endothelial function by increasing superoxide production and decreasing the activity of nitric oxide synthase. Homocysteine could be a starting point for the development of atherosclerosis by disturbing vascular permeability, damaging the inner lining of the arteries and promoting blood clots. Slidroside extracted from *Rhodiola* protect rats aortas against homocysteine-induce impairment of endothelium by inhibiting NOX2-dependent ROS overproduction. These results suggest that salidroside significantly inhibit ROS overproduction associated with vascular dysfunction, a common pathological process in hypertension and diabetes.[11]

k) Effect on Binge eating and Anorexia

Binge eating (BE) and Anorexia Nervosa are official eating disorders. Binge eating appears to be characterized by extreme overeating without subsequent purging episodes, usually secretive, and filled with shame.[37] Topiramate or sibutramine are medications that have been suggested to reduce BE. However, their uses are associated with a variety of adverse side effects which causes serious problems, such as cardiovascular disorder and stroke. As a result they have been withdrawn from the market in many European countries. Since stress is a key factor in BE, a reduction of stress response might show an effective mechanism for the treatment of BE. Therefore, due to its anti-stress properties, the effect of Slidroside, an active principle of the dry extract of *R. rosea*, was evaluated for treatment of BE. Studies have shown that Salidroside abolishes BE by suppressing the activation of hypothalamic-pituitary- adrenal (HPA) axis, leading to a reduction of serum corticosterone flowing chronic treatment.[1]

Furthermore, new evidence shows that *R. rosea* may cancel out the anorexia (out of control dieting), another troubling manifestation of stress.

Eating disorders are associated with stress responses depending on the intensity of stress itself; moderate stressors stimulate eating while acute stressors, which cause high levels of CRF (corticotrophin-releasing factors), induce anorexia. In particular, considerable evidence suggests a role for endogenous brain CRF system in appetite regulation and the cause of eating disorder. At doses of 15 and 20mg/kg, *Rhodiola* extract significantly inhibits the anorexia effects of stress within 60 minutes after a single oral administration of *R. rosea* extract.[38] Therefore, the different effects evoked by *R. rosea* on eating behavior could be attributed to its ability to modulate the activation of several components of stress-response system rather than a direct effect on orexigenic or anorexigenic mechanisms.[1]

l) Effect on Parkinson's Disease

Parkinson's disease (PD) is a chronic and progressive disorder of the nervous system that affects movements of the body and the symptoms continue and worsen over the time. Parkinson's primarily affects neurons in the area of the brain called substantia nigra. Cells within the substantia produce and release dopamine, a neurotransmitter that controls the movement and balance. In patients suffering from Parkinson's, the amount of dopamine produced in the brain decreases. The shaking or tremor may begin to interfere with the daily activities of the PD patients. As these symptoms become more pronounced, patients may have difficulty walking, talking or performing other simple tasks. Although

there is no cure, there are treatment options such as medication and surgery to control the symptoms.[39]

The new plant preparation Phytomix-40(PM-40) is developed for the treatment of Parkinson's disease. Phytomix (PM-40) is a mixture of natural extracts of 40 medical plants, including extracts of *R. rosea*, Eleutherococcus, ginseng, and other adaptogens with neuroprotective properties. Animal experiments demonstrated that PM-40 had a low toxicity. The neuroprotective plant adaptogen can be used in complex therapy for the Parkinson's disease for improving its efficacy. Oral administration of 10% solution of Phytomix-40 to mice with MPTP-induced Parkinson's syndrome reduces the severity of rigidity and increase motor activity. The preparation normalized immunobiological parameters in PD patients and relieved the clinical symptom of the disease. The mechanism of action of PM-40 contributes to the recovery of the dopamine synthesis by healing of damaged neurons. PM-40 can be used with the combination of other standard antiparkinsonian drugs in order to improve their clinical effects and minimize side effects of Parkinson's medication.[39]

m) Overview of toxicological and safety data

Through the doses administered in clinical trials, there is no report of serious side effects that could be attributed to the extract of *Rhodiola rosea*. The normal usage of R. rosea is safe, however, it is important to consider that R. rosea, a strong adaptogenic and tonic herb, might have an addictive effect with other substances exhibiting stimulant properties (such as caffeine).[40]

Continuous daily use of *R. rosea* for days and months is followed by an interval with no supplementation (three weeks "on" and one week "off"). This clinical recommendation helps avoid possible side effects at higher dosages such as insomnia, irritability, dizziness, dry month, and allergy (unspecified).[29]

The most commonly used standardized extract has a minimum of 3% rosavin and 1% salidroside. The typical daily dose for chronic administration extracts range from 100-170 mg per day when standardized for 2.6% rosavin. Evidence on the safety and appropriateness of *R. rosea* supplementation during pregnancy and lactation has not been established.[14]

IV. Conclusions

Rhodiola rosea, which is also known as the golden root, is one of the most studied *Rhodiola* species. As an adaptogen, many health benefits are related to *Rhodiola* drug extracts due to their balancing and regulatory effects. Significant antioxidant activities have been documented for various *Rhodiola* species extracts. In Russian and Chinese folk medicine, the plant is used for stimulating the nervous system and decreasing mental and physical fatigue. It has been shown in pharmacological investigations that, *R. rosea* possess antioxidant, antiaging, anti-cancer and anti-cardiovascular disease properties. As a dietary supplement, numerous preparations of extracts are used worldwide including teas, homeopathic preparations and tinctures as well as standardized extract. *Rhodiola rosea* has enormous traditional and pharmacological use in supporting mood and cognitive function.

Rhodiola rosea is a versatile, safe and easily accessible plant which offers resistance to the physical, chemical and biological stressors without interacting with other food or drugs. The remarkable therapeutic effects of this plant in prevention and treatment of variety of human diseases, makes this plant very valuable for further investigation in the area of pharmaceutical industries.

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Drug Related Problems that Occurred in Patient Sepsis Macrovascular Disease Complications General Hospital Treatment Room Central of the Army (Army Hospital) Gatot Subroto

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Abstract- Sepsis is a systemic inflammatory response syndrome: clinical inflammatory response to disturbances that cause infection or not infection1. Patient entered in the Emergency Room (ER) 8 October 2014, the diagnosis of sepsis patient ec furnier gangrene (scrotum), Haematemesis ec gastropathy uremikum, CKD stage V on HD with anemia, metabolic acidosis, CHF fc II, type II diabetes mellitus, hypertension, hepatitis B therapy received treatment for at GatotSubroto Army Hospital namely, Lasix, D40%, Insulin (Novorapid, Humalog), Meropenem, Levofloxacin, Farmadol, Omeprazole, Transamin, Vitamin K, Sodium Bicarbonate, Calcium Carbonate, Folic Acid, Vitamin B12, Valsartan, Amlodipine, Transfusion Package Red Cells (PRC), Triofusin e 1000, Tramal, Ca Gluconate, Metronidazole, Cefoperazone Sulbactam, Albumin transfusion, transfusion Fresh Frozen Plasma (FFP), the result of monitoring drug therapy patient obtained their multiple DRPs *(Drug Related Problems),* correlation between therapy with disease, Selection of appropriate medication, dosage regimen, interactions and contraindications.

Keywords: sepsis, complications, gatot subroto army hospital.

GJMR-B Classification : NLMC Code: WF 360

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Abdullah [α], Diana Laila Ramatillah [σ] & Aprilita Rinayanti Eff [ρ]

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I. Introduction

Sepsis presence of pathogenic microorganisms or their toxins in the blood and other tissues, Systemic inflammatory response syndrome: clinical inflammatory response to disturbances that cause infection or not infection[1]. Responses appear in the form of ≥ 2 the following conditions: temperature > 38°C or < 36°C, frequency heart rate > 90 beats/min, respiratory frequency > 20 breaths/min, the white blood cells > 12000 cells/mL or < 4000 cells/mL or > 10% in the form of immature[1]. Severe sepsis: sepsis associated with organ dysfunction, septic shock: sepsis with hypotension simultaneously the presence of perfusion abnormalities[1]. Causinga frequent site of infection sepsis (21-68% Respiratory tract, urinary Channels 14-18%, 14-22% Intra Abdominal cavity): Sepsis by gram-negative bacteria (approximately 38% of the incidence of sepsis) *Escherichia coli* and *Pseudomonas aeruginosa* is bacteria the most frequently isolated in sepsis, Gram-positive (40%): *Staphylococcus aureus, Streptococcus pneumoniae, Staphylococcus coagulase negative and, Enterococcus.* The cause of fungal sepsis (17%): *Candida albicans* frequently causes sepsis in hospital patient[1].

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II. Clinical Evaluation

While in hospital the patient was given medication therapy are: Lasix 18 ampoules / 24 hours up to 1,5cc / hour because Lasix is very effective to cope with edema administration IVFD to the effect that faster with gradual dose. D40% 2 flacon / 3 hours up to 16 cc / hour as a replacement fluid and energizing, glucose solution is administered in treatment with Calcium, Sodium Bicarbonate, and Insulin for the emergency management of hyperkalemia. Triofusin e 1000 500 cc / 24 hours for obtaining calories and electrolytes needed through total and partial parenteral nutrition in patient with diabetes needs to be done to monitor blood sugar levels[2].

Insulin 0,5 units / hour in NaCl 0,9% for patient undergoing surgery and require insulin infusion intravenously for 12 hours or more, the speed of infusion of insulin if blood sugar < 4 mmol / liter is given 0,5 units / hour for onward adjusted to the patient's clinical condition, after the patient began to eat and drink, give insulin subcutaneously use multiples of 4 units of insulin applies if < 200 mg / dL = 0 units, 201 - 250 mg / dL = 4 units, 251 - 300 mg / dL = 8 units, 301 - 350 mg / dL = 12 units, > 351 mg / dL = 16 units beyond[2].

Meropenem 1 gram 3 times daily in this case as empirical therapy, while awaiting culture results come out, Meropenem be an option because of its broad spectrum for infection of gram-positive and gram-negative, aerobic and anaerobic, tousesoshould be based on culture results, while for patient impaired liver function and impaired renal function the dose and treatment regiment adapted to the patient's clinical condition, Cefoperazone Sulbactam 2 times a day 1 gram for gram-positive need for strong administration drip for 1 hour, 1 time a day Levofloxacin 500 mg is indicated for patient who have urinary tract infections are caused by infection with gram-positive and gram-

negative, and dose adjustment regiment not promising therapy for patient with impaired renal function and liver function disorders, Metronidazole 500 mg 3 times also used for gynecological surgery sepsis with major activity against anaerobic bacteria colonic[2,3].

Omeprazole 40 mg 2 times a day as therapy uremikum gastropathy and gastric ulcers and reduction during general anesthesia (acid aspiration prophylaxis) is given 40 mg in the afternoon and one day prior to surgery and then 40 mg 2-6 hours before surgery, Inpepsa suspension effective in treating ulcers stomach, protecting the mucosa from acid-pepsin in gastric ulcer timing of 2 gram 2 times a day (morning and before bed at night) or 1 gram 4 times a day 1 hour before meals and before bed at night, given for 4-6 weeks[2].

Tramal 100 mg 3 times daily for pain, Farmadol given 3 times 1 gram to address moderate pain till mild postoperative pain and fever, Ca Gluconate quickly can lead to vasodilation of blood vessels, decrease in blood pressure, bradycardia and cardiac arrhythmias, and even can cause cardiac arrest therefore IV administration either bolus or continuous need to monitor blood pressure and pulse of this reaction is due to a decrease in potassium drastically in rapid decrease in potassium will result in a decrease in contractile muscle cells, including heart muscle cells resulting in a decrease in pulse and vasodilatation[2,3].

Transamin injection of 500 mg 3 times a day is given to inhibit fibrinolysis so it can be useful to prevent bleeding, is used in patient with mild renal function disorders at the recommended dose reduction, whereas for patient with severe renal function impairment and avoid use in patient with impaired function liver needs to be monitored, Vitamin K 3 times daily 10 mg is needed for the production of blood clotting factors, for patient impaired liver function may have deficiencies vitamin K [2,3].

Sodium Bicarbonate 1 gram 3 times daily to cope with metabolic acidosis, in severe cases can be administered by intravenous Sodium Bicarbonate, Calcium Carbonate 500 mg 3 times daily is used as a phosphate binder in the treatment of hyperphosphatemia in renal failure complications[2,3].

Folic Acid 15 mg 1 time a day is required for nucleoprotein synthesis and maintenance of normal erythropoiesis, folic acid stimulates the production of red blood cells and white blood cells, Vitamin B_{12} 50 mg 3 times daily is important for growth, cell reproduction hematopoiesis, and nucleoprotein synthesis and meilin, Vitamin B_{12} also plays a role in the formation of red blood cells through the activity of folic acid coenzyme[2].

Valsartan 160 mg 1 time a day for the treatment of hypertension that can be combined with other antihypertensives, Valsartan may be given to patient with heart failure, patient with hemodialysis, the patient is low in sodium, this group does not inhibit the breakdown of bradykinin that does not cause a dry cough, Amlodipine1 time a day 10 mg as antihypertensive, how it works inhibits calcium ion influx through the slow channel membran active cell, thereby affecting cardiac myocardial cells, and vascular smooth muscle cells and reducethe ability of myocardial contraction, the formation and propagation of electrical impulses in the heart, and systemic or coronary vascular tone, Amlodipine did not reduce myocardial contractility and does not cause deterioration in heart failure with a longer tenure so that it can be given once a day[2,3].

PRC 500 cc of blood transfusion aims to improve the oxygenation of tissues and organs with a target of 8 g / dL, transfusion Albumin to overcome the shortage of Albumin in the body, can lead to instability Albumin shortage of water in the blood plasma, so that the blood volume is unstable and undergo body hoarding fluid which is often characterized by swelling, Albumin also act as transport in the body, including some elements of drugs and assist in the formation of a new body tissue, addition of Albumin transfusion transfusion patient also in Fresh Frozen Plasma (FFP) contains all plasma proteins that are fresh frozen plasma the goal is to reach 30% of normal clotting factor concentrations[2].

III. Dosage and how to use

Lasix initial dose of 250 mg to 4 mg / min for 1 hour, the dose may be increased to 1 grams can be repeated every 24 hours. D40% given 1-3 liters / day, Triofusin e 1000 500 cc for 24 hours, use multiples of 4 units of insulin effect, if the blood glucose < 200 mg / dL = 0 unit, 201 - 250 mg / dL = 4 units, 251 – 300 mg / dL = 8 units, 301 – 350 mg / dL = 12 units, > 351 mg / dL = 16 units onwards. Meropenem 250 mg every 24 hours after the HD 500 mg every 8 hours, Cefoperazone Sulbactam 500 mg every 12 hours up to 1 gram a day, the initial dose after Hemodialysis Levofloxacin 500 mg to 250 mg every subsequent 48 hours for 7 – 14 days, Metronidazole 500 mg every 8 hours, Omeprazole 40 mg every 24 hours, Ranitidine 50 mg every 6-8 hours, Tramal 50 - 100 mg every 4 - 6 hours, Farmadol 1 g every 4 - 6 hours maximum 4 grams / day, Cagluconate 10 ml (2, 25 mmol) - 40 ml (40 mmol) of 10% / day, Transamin injection of 500 mg - 1 g every 8 hours, 10 mg Vitamin K Injection for 24 hours[2,3].

Valsartan 40 mg every 12 hours dose adjustment 80 - 160 mg every 12 hours, Amlodipine 5 mg - 10 mg every 24 hours, Inpepsa suspension 2 - 4 g every 12 hours for 4 - 6 weeks up to 8 grams, 500 mg Sodium Bicarbonate rapid dehydration every 3-4 hours, later every 12 hours, Calcium Carbonate 500 mg every 8 hours, Paracetamol 500 mg - 1 grams every 4 - 6 hours maximum 4 grams, Folic Acid 5 mg every 24 hours depending on the disease Basically, Vitamin B12 50 - 150 mcg or more given between meals every 8 hours[2,3].

Blood transfusion Package Red Cells (PRC), Albumin transfusion, Transfusion of Fresh Frozen Plasma (FFP)

IV. Laboratory Results

Examination	Abnormal values	Normal value
	Routine Hematology	
Hemoglobin	7,8 *	13-18 g / dl
Hematocrit	23 *	40-52%
Erythrocyte	2,9 *	4,3 to 6,0 million / mL
Leukosite	14080 *	4800-10800µL
MCV	78 *	80-96fL
	Coagulation	
PT	13,4 *	10,2 to 12,2 seconds
APTT	48,4 *	29 to 40,2 seconds
	Clinical Chemistry blood gas analysis	
pH	7,477 *	7,37 to 7,45
pCO_2	23,2 *	33-44mmHg
pO_2	42,7 *	71-104mmhg
Bicarbonate (HCO_3)	17,3 *	22-29mmol / L
Base excess (BE)	-4,6	(-2) -3mmol / L
O_2 saturation	82,7 *	94-98%
Albumin	24 *	3,8 to 5,1 g / dL
Urea	172 *	20-50mg / dl
Creatinine	11,6 *	0,5-1,5mg / dl
Calcium (Ca)	7,2 *	8,6-10,3mg / dl
GDS	179 *	<140mg / dl
Sodium (Na)	134 *	135-147mmol / L

Calculations Estimate Creatinine Clearance (CrCl) based on the Cockcroft-Gault[4].

$$\text{CrCl} = \frac{\text{Weight (kg)} \times (140\text{-age})}{72 \times (\text{Cs}) \text{ cr (mg \%)}}$$

$$\text{CrCl} = \frac{80 \text{ (kg)} \times (140\text{-}49)}{72 \times (11,\ 6 \text{ mg \%})}$$

$$\text{CrCl} = 8,\ 71 \text{ mL/min}$$

V. Drug Related Problems (DRPs)

1. Correlation between therapy with disease

There was a clinical condition was not treated, namely hepatitis B based on ISO book Pharmacotherapy should be given hepatitis B vaccine therapy (HBIG).

Pharmacist Intervention: The dose of hepatitis B vaccine (HBIG) is usually 0,06 ml / kg IM administered in a single dose for 14 days[1].

2. Selection of appropriate medication

Selection of the drug wasnot safe for the patient's condition and dosing indispensable for these patient (CKD stage V) because some drugs can not be in clean when hemodialysis.

Pharmacist Intervention: Patient with stage V renal failure need dose adjustments are based on those calculations the dose should be decreased Creatinine Clearance Estimate of laboratory values[5].

3. Dose regimen

The dose, frequency and route of administration did not consider the effectiveness, safety, comfort, and not in accordance with the patient's condition? Meropenem 1 gram 3 times daily, Cefoperazone Sulbactam 2 times daily 1 gram, 1 time a day Levofloxacin 500 mg, Valsartan 160 mg 1 time a day

Pharmacist Intervention: Use of drugs tailored to the patient's clinical condition; Meropenem for ClCr < 10 mL / min to 250 mg for 24 hours, after hemodialysis given 500 mgevery 8 hours, Levofloxacin Cr 10-19 mL / min after hemodialysis therapy dose of 500 mg to 250 mg subsequently every 4 hours for 7 - 14 days, Cefoperazone Sulbactam ClCr <15 mL / min therapeutic dose of 0,5 grams for 12 hours up to 1 gram / day, Valsartan as an antihypertensive drug, administered dose of 160 mg lowered to 40 mg1 time / day in patient with Chronic Kidney Disease (CKD) on Hemodialysis[5].

4. Interactions and contraindications

There were interactions between drugs with drug, Potassium Chloride + Valsartan need for dose adjustment may increase potassium in the blood, Tramadol + Meropenem + Levofloxacin can affect the central nervous system resulting in convulsions, Insulin + Sucralfate should be avoided or dose adjustments of Insulin because of Sucralfate suspension containing carbohydrates so can interfere with blood glucose levels, Calcium Carbonate + Sucralfate + Amlodipine, a Calcium Carbonate can inhibit the action Sucralfate and Amlodipine, Lasix + Cefoperazone concurrent use can worsen kidney function requires monitoring of renal function[6].

Pharmacist Intervention: Potassium Chloride + Valsartan discontinued due to the use of potassium normal laboratory values, Tramadol + Meropenem + Levofloxasin because this drug is needed in the treatment of patient were advised to use Meropenem precedence because T½ of Meropenem shorter that 1 hour of tramadol with T½ 6 hours, while for Levofloxasin given every 48 hours for patient with CKD condition that can be given after use of Tramadol, Insulin + Sucralfate improved insulin dosage based on blood glucose levels. Calcium Carbonate + Sucralfate + Amlodipine, Amlodipine use of precedence by chewing and swallowed to accelerate the absorption of Calcium Carbonate inthe next administration and Sucralfate given within 1 hour after administration of Calcium Carbonate, Lasix + Cefoperazone the use of Lasix precedence 30-60 minutes of use Cefoperazone[1,2,3,6,7].

VI. Conclusion

Based on the assessment of the use of drugs were used, it can be concluded that, in patients with a diagnosis of sepsis macrovascular disease complications, found the presence of some DRPs (Drug Related Problems), correlation between therapy with disease, Selection of appropriate medication, dosage regimen, interactions and contraindications.

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Chemistry, Pharmacology and Medicinal Property of Frankincense (Boswellia Species): From the Selection of Traditional Applications to the Novel Phytotherapy for the Prevention and Treatment of Serious Diseases

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

Abstract- Frankincense, the resinous extract from the trees of the genus Boswellia, has been used for centuries in ceremonial, cosmetic, cultural and as a traditional medicine to treat a variety of ailments especially inflammatory diseases including asthma, arthritis, cerebral edema, chronic pain syndrome, chronic bowel diseases, cancer and some other illnesses. Boswellic acids are the active compounds of frankincense and AKBA (3-O-acetyl-11- keto-**β**-boswellic acid) is the most important and effective acid among them. Some studies have shown that the use of frankincense can also improve the learning and enhance the memory in animals and human. It seems that frankincense might have a potential ability to be used as an alternative natural medicine not only for chronic and inflammatory diseases but also for the patients with brain and memory disorders.

Keywords: boswellia species, anti-inflammatory, chronic diseases, cancer, memory enhancement.

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Chemistry, Pharmacology and Medicinal Property of Frankincense (Boswellia Species): From the Selection of Traditional Applications to the Novel Phytotherapy for the Prevention and Treatment of Serious Diseases

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

Abstract- **Frankincense, the resinous extract from the trees of the genus Boswellia, has been used for centuries in ceremonial, cosmetic, cultural and as a traditional medicine to treat a variety of ailments especially inflammatory diseases including asthma, arthritis, cerebral edema, chronic pain syndrome, chronic bowel diseases, cancer and some other illnesses. Boswellic acids are the active compounds of frankincense and AKBA (3-O-acetyl-11- keto-β-boswellic acid) is the most important and effective acid among them. Some studies have shown that the use of frankincense can also improve the learning and enhance the memory in animals and human. It seems that frankincense might have a potential ability to be used as an alternative natural medicine not only for chronic and inflammatory diseases but also for the patients with brain and memory disorders.**

Keywords: boswellia species, anti-inflammatory, chronic diseases, cancer, memory enhancement.

I. Introduction

Frankincense is a French word, meaning "pure incense". It is popularly known as Indian olibanum, Salai guggal, Loban, or Kundur. It has been used as an incense and fumigating preparation for religious rituals, in cultural ceremonies and as a traditional remedy for treating various diseases.[1] The oleogum resins are secreted by trees of the Boswellia species which are tropical, deciduous trees and usually grow as small trees or shrubs with the limited natural growing range.[2] The growing of the trees has been extended by cultivation to meet the worldwide demand.[3] The resin is obtained by making scrapes in the trunk of the various Boswellia species (Burseraceae), and later collecting the dried resin gums from the trees.[2,4] The good quality of resin is produced only for three years and after this period, the quality of collected resin decreases significantly, then the tree should be left to rest for some years after the harvesting period.[5]

Olibanum is produced mainly by four species from different regions such as Boswellia serrata from India, Boswellia carterii from East Africa and China, Boswellia frereana from Northeast Africa (Somalia) and Boswellia sacra from Middle East.[6,7] Today the most traded frankincense is produced in Oman, Yemen and Somalia.[3]

Since ancient times, Frankincense has been used in many countries such as Africa, China, India and Middle East countries for the prevention and treatment of various illnesses especially chronic inflammatory diseases.[2,8] In Indian system of medicine, Frankincense (salai guggal) has been used as an anti-inflammatory, anti-arthritic, anti-proliferative and analgesic agent for the treatment of related diseases.[9] In Traditional Chinese Medicine (TCM), frankincense of Boswellia carterii is commonly used as a remedy for improving blood circulation and as relieving pain in leprosy, gonorrhea and cancer patients.[10]

In the last decade, the use of olibanum has become more popular in European countries for the treatment of variety of chronic inflammatory problems such as arthritis, chronic bowel diseases, asthma, peritumoral brain edema and other diseases.[11]

The mechanism of anti-inflammatory activity of the Boswellia extract is due to the boswellic acids which are the active principals of frankincense. The chemical structure of boswellic acids closely resembles steroids,[9] which their actions are different than painkillers or NSAID (non-steroid anti-inflammatory drug) and is related to the component of the immune system and the inhibition of 5-lipoxygenase.[11]

II. Composition

There are many different compounds found in a variety of Boswellia species.[11] The composition of the essential oil and other contents changes from species to species, and differs depending on the climate, harvest conditions and geographical locations. [11,12]

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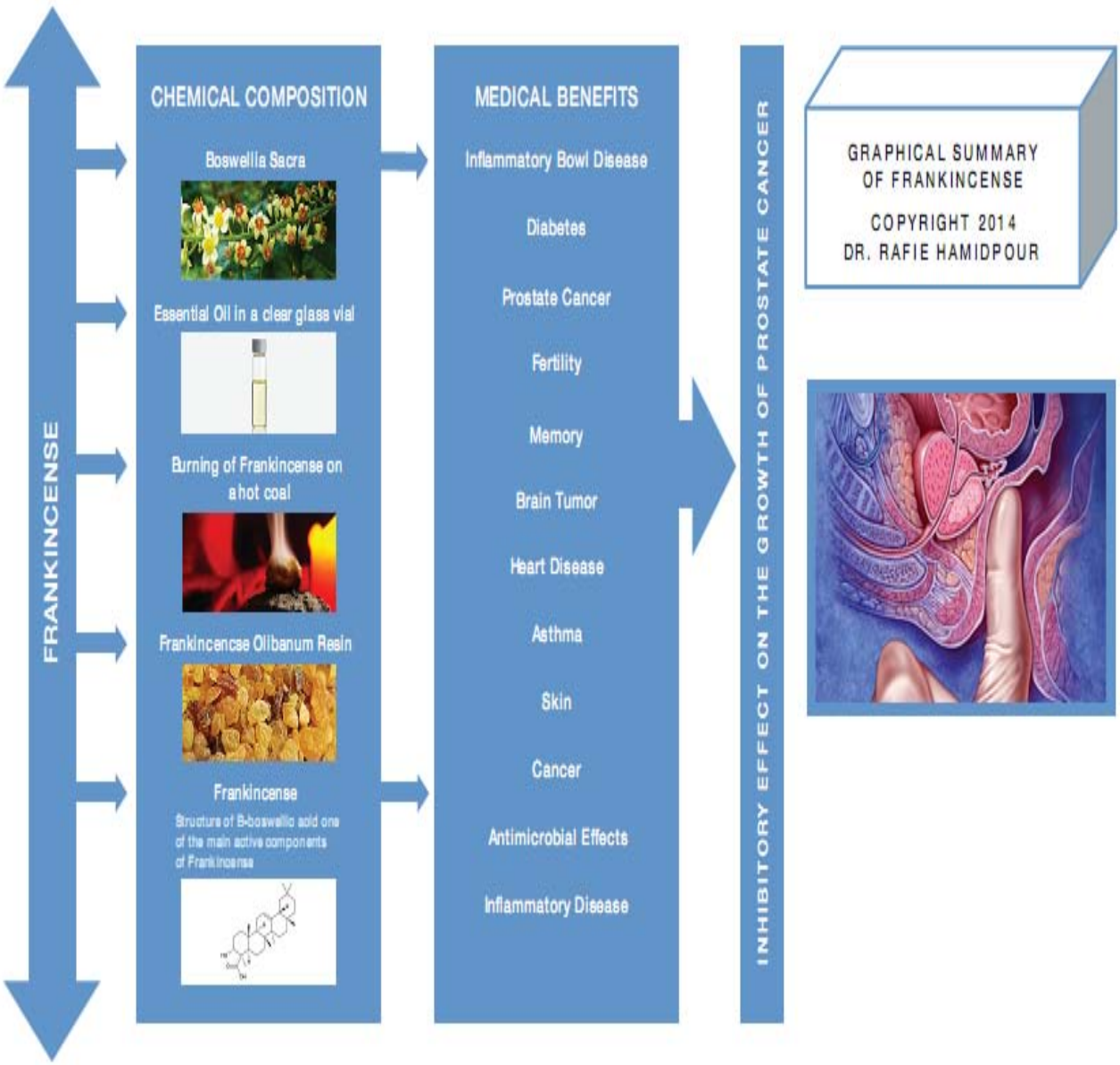
Frankincense is reported to contain 60-85% resins (mixtures of terpenes), 6-30% gums (mixture of polysaccharides) and 5-9% essential oil. [13] Resin portion is composed of pentacyclic triterpenes which boswellic acid is the active functional group.[14] Gum portion is consisting of pentose and hexose sugar with some oxidizing and digestive enzymes. The essential oil is the mixture of monoterpenes, diterpenes and sesquiterpenes.[14]

In a study was reported that the resinous part of Boswellia serrata contains terpenes: monoterpenes (α-thujone); diterpenes (macrocyclic diterpenoids such as incensole, incensole oxide, iso-incensole oxide, a diterpene alcohol [serratol]); triterpenes (such as α- and β-amyrins); pentacyclic triterpenic acids (boswellic acids); tetracyclic triterpenic acids (tirucall-8, 24-dien 21-oic acids).[5]

Boswellic acids with the molecular formulas of C_{32} H_{52} O_4 are the main active component of frankincense.[12] The four major boswellic acids (pentacyclic triterpenic acids) found in frankincense are: β-boswellic acid (BA), acetyl-β-boswellic acid (ABA), 11-keto-β- boswellic (KBA) acid and 3-O-acetyl-11-keto-β boswellic acid (AKBA) which they have shown to be responsible for the inhibition of Pro-inflammatory enzymes.[5] Among these four boswellic acids, acetyl-11-keto-β boswellic acid (AKBA) is the most important inhibitor of an enzyme called 5-lipoxygenase which is responsible for inflammation.[5]

AKBA has shown to be effective against a large number of inflammatory diseases such as arthritis, bronchial asthma, chronic colitis, ulcerative colitis, Crohn's disease and cancer.[15] The mechanism of the action is due to the binding of AKBA to 5-lipoxygenase in a calcium-dependent and reversible and act as a non-redox type, none-competitive inhibitor.[16]



III. Inflammatory Diseases

Inflammation, generally is the response of the body tissues to irritation, injuries, infections, or disorders of the immune system (auto-immune diseases), which is characterized by pain, redness, swelling and sometimes loss of function.[11]

Leukotrienes are small mediator chemicals produced by cells in the body which can cause inflammation by promoting free radical damages, autoimmune responses, cell adhesion, and migration of the inflammatory producing cells to the inflamed area.[17]

Many inflammatory diseases can be caused by leukotrienes including asthma, colitis, rheumatism, arthritis and psoriasis. [17] Boswellia has shown to be the specific inhibitor of leukotrienes. Boswellia acts by blocking the synthesis of leukotrienes and therefore inhibiting the inflammation and shrinking the inflamed tissue which is the primary cause of pain and discomfort in many cases.[17]

Frankincense has shown to be effective in treating various inflammatory diseases and based on data obtained from the experiments done in vitro and vivo, boswellic acids are assumed to be the pharmacological active principals of frankincense which are responsible for the anti-inflammatory and anti-tumorigenic actions.[18] In a study the anti-inflammatory effects of the extracts and powder of frankincense on the plaqueinduced gingivitis showed the improvement of inflammation of periodontium after using the extract and powder of the frankincense.[19]

a) Heart Disease

Frankincense, the oleogum resins of Boswellia species have been used in traditional medicine for the treatment of various inflammatory diseases and today they are used as a complementary and alternative medicine for the treatment of some chronic inflammatory diseases.[20]

Atherosclerosis is the build up of plaque on the inside of blood vessels, causing the hardening of the arteries. Atherosclerosis is the major cause of coronary heart disease and it has been found to be linked with inflammation.[20] All the data clearly indicates that AKBA reduces chronic inflammation through the inhibition of NF-κB (nuclear transcription factor) system which is a very important factor in developing and progress of chronic inflammatory diseases.[20] Therefore therapeutic approaches targeting this transcription factor to treat chronic inflammation in atherosclerosis could be developed.[20]

b) Asthma

Frankincense traditionally has been valued for its effect on the respiratory system and has been used in steam inhalations, baths and massages to treat cough, catarrh, bronchitis, and asthma.[9] Boswellic acids found in frankincense have shown to be responsible for the inhibition of leukotrienes biosynthesis and therefore can reduce and prevent the inflammation in many chronic inflammatory diseases like asthma.[21] In a study several patients with chronic bronchial asthma were treated with the Boswellia serrata preparation of 300 mg thrice daily for a six-week period. The improvement of the disease was obvious for 70% of the patients by disappearance of physical symptoms and signs such as dyspnoea (Difficulty in breathing), rhonchi (hissing lung sound) and number of attacks. The data show a definite role of gum resin of Boswellia serrata in the treatment of bronchial asthma.[21]

c) Skin

Studies have shown that the presence of Boswellia serrata extract reduces the redness and irritation in the skin and produces an even skin tone.[22] In China frankincense has been used as skin remedies for bruises and infected sores.[4] The extracts of Boswellia family have shown to have a soothing effect for irritated skin and that is caused by the pentacyclic triterpene (steroid-like) structure shared in different Boswellic acid compounds.[22]

To allow for easy incorporation of the extract of Boswellia, containing the boswellic acids and its derivatives, the extract needs to be dissolved or dispersed in a suitable carrier such as fatty alcohols, or fatty acids which help to incorporate the extract or acid into compositions suitable for use on skin or hair and improves the stability of products containing the extract.[22] In addition, Acetyl-11-keto-β boswellic acid (AKBA) is reported to be an effective topical agent to soften facial lines and relax the skin.[16]

IV. Inflammatory Bowel Disease

Inflammatory bowel diseases (IBD) refer to the inflammation of intestines and relate to two chronic diseases, ulcerative colitis (UC) and Crohn's disease (CD). Although the exact cause of IBD is still not clear, but two factors are considered to be effective in occurring the disease. First is the immune dysregulation which is caused by genetic or environmental factors and second is abnormal gastrointestinal tract luminal factors such as microorganisms of GI tracts, oxidative stress, and defects in the GI mucosal barrier which allows the penetration of luminal factors into mucosa.[23]

The leukotrienes play an important role for keeping inflammation active in chronic inflammatory diseases of the colon such as ulcerative colitis. Boswellic acids, which are the active ingredients of the gum resin of Boswellia species have been shown to be specific, non competitive inhibitors of 5-lipoxygenase, the key enzyme of leukotrienes.[24,25]

The preparation of the gum resin of Boswellia serrata has shown to be effective in the treatment of chronic colitis with the smallest amount of side

effects.[24] In traditional Iranian medicine (TIM), oleogum resin of Boswellia serrata and Boswellia carterii is known for reducing the inflammation and one of the efficacious remedy for the treatment of IBD. In addition to anti-inflammatory effects, Boswellia has shown to have wound healing, antiulcer, and anti-diarrheal properties too.[23]

In a study, was found that the use of the gum resin of Boswellia serrata was effective to induce the remission in about 80% of the patients with ulcer colitis grade II and III, when was applied 350 mg three times a day over period of six weeks. The study suggests that the effectiveness of frankincense if is not better, at least is similar to the treatment with sulfasalazine which is a chemical drug used in the treatment of inflammatory bowel diseases.[25]

a) Cancer

Plants are rich sources of antitumor compounds. Oleogum resins from various Boswellia species contain triterpenoids with antitumor properties.[26] In a report, the anti tumor activities of the four triterpenic acids (BA, ABA, KBA and AKBA) isolated from the gum resin of Boswellia serrata were studied and it was found that these acids inhibited the synthesis of DNA, RNA and protein in human leukemia HL-60 cells in a dose dependent system. Among them AKBA induced the most pronounced inhibitory effect on DNA, RNA and protein synthesis in which the effect on DNA synthesis was found to be irreversible. This compound significantly inhibited the cellular growth of HL-60 cells, but did not affect cell viability.[24]

The studies have shown that boswellic acids are potent apoptotic agents to cancer cells. The boswellic acid acetate seems to induce apoptosis in six human myeloid leukemia cell lines through a Caspase-mediated pathway which is activated by the induction of the death receptors 4 and 5 (DR4, DR5).[27] The anticancer activity of AKBA is attributed to the inhibitory effect on the lipoxygenases leading to the inhibition of cell proliferation and induction of apoptosis in tumor cells.[15]

b) Prostate Cancer

It has been shown in several studies that pentacyclic triterpenoids found in Boswellia serrata have inhibitory effect on the growth of prostate cancer cells.[26] Among boswellic acids, Acetyl-11-keto-β-boswellic acid (AKBA) has special inhibitory effect in prostate cancer by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis.[5] Also tirucallic acids isolated from the oleogum resin of Boswellia carterii work as an effective Akt inhibitors which apply cytotoxic effects in human prostate cancer cell lines in vitro and vivo.[26]

Akt is a serine/threonine protein kinase which has an important role in multiple cellular processes such as cell proliferation, apoptosis, transcription and cell migration. Akt1 has been associated as a major factor in many types of cancer since it can block apoptosis and promote the survival of the cell.[26]

c) Brain Tumor

Brain cancer is a condition in which malignant tumors develop within the brain. These tumors are fast growing and invade surrounding tissues. The surgical removal of brain tumors is a hard and detailed procedure and in many cases the complete removal of the tumor is not possible because of the size, type and location of the tumor. For these reasons, the average survival of brain tumor patients is only about nine months even after the treatment of surgery and radiotherapy are combined.[28] In addition the use of chemotherapy is able to prolong the survival of only about 10% of the patients.[28] In patients with malignant brain tumors, highly active forms of leukotrienes and other inflammatory mediators are produced in the brain and around tumors, causing localized fluid build-ups and damages to the healthy nerve cells.[29]

The impact of Boswellia serrata, with its anti inflammatory effect has been studied in patients with brain tumors.[29] An ethanolic extract from the gum resin of Boswellia serrata contains the boswellic acids which the study have shown after the application of this preparation which is called phytopharmacon H15 for the period of seven days a reduction of the peritumoural brain edema of 22 to 48% was observed. In contrast to the cells of untreated patients, the cells of the treated tumor tissue show no tendency to proliferate within two weeks.[28]

The report on patients with malignant glioma showed that the use of 3600mg/ day of Boswellia extract (60% boswellic acids), seven days prior to surgery caused decrease of the fluid around the tumor with average of 30% in 8 of the 12 patients and the signs of brain damage decreased during the treatment.[29] Recently the detailed study in patients with malignant cerebral tumors who were receiving radiotherapy plus certain amount of Boswellia extract, showed that after the end of radiotherapy the 75% reduction of cerebral edema was observed in 60% of the patients receiving Boswellia extract. The study also shows the ratio of tumor over volume decreased in these patients, suggesting anti-tumor effect in addition to the anti-edema activity.[29]

d) Diabetes

The effect of Boswellia has been known on wide variety of diseases, including inflammatory diseases and diabetes mellitus.[30] The study has shown that the oral administration of aqueous extract of the leaves and roots of Boswellia glabra decreased the blood glucose level in diabetic patients. The continuation of the use of leaf and root extract for 28 days showed a decrease in serum glucose, cholesterol, triglyceride, urea and

creatinine levels and enzyme activities in addition to significant hypoglycemic effects.[30]

Type I diabetes is an autoimmune disease in which a chronic inflammatory process finally causes beta-cell death and insulin deficiency. Extracts from gum resin of Boswellia serrata have been shown to possess anti-inflammatory properties especially by targeting factors or mediators related to autoimmune diseases.[31] The study shows that Boswellia extract has anti-diabetic effects and could prevent complications of diabetes in the kidneys and liver.[32]

e) Antimicrobial

The essential oil isolated from the oleogum resin of Boswellia carterii has shown to have antimicrobial activities against various microorganisms such as fungi, Gram-positive and Gram-negative bacterial strains.[23] In a study the antibacterial activity of boswellic acids were tested in vitro on a group of clinically significant Gram-positive and Gram-negative bacteria. Among the boswellic acids Acetyl-11-keto-beta-Boswellic acid (AKBA) was the most active inhibitor of bacterial pathogens. However the activity of AKBA was limited to Gram-positive bacteria.[33] The resistance of Gram-negative bacteria to lipophilic AKBA might be as a result of the presence of lipophilic outer membrane which is composed of primarily of lipopolysaccharide molecules and forms a hydrophilic permeability barrier providing protection against the effects of highly hydrophobic compounds.[33]

Biofilms are multilayered community of bacterial cells. Staphylococci are known to form biofilms on an implanted medical device or damaged tissues which are difficult to disrupt. The infections caused by biofilms are difficult to treat due to their inherent antibiotic resistance.[33] In a study, AKBA effectively inhibited the staphylococcal biofilm and also reduced the performed biofilm of these bacterial pathogens. This is the report which shows that AKBA can prevent as well as reduce the S. aureus and S. epidermidis generated biofilms.[33] AKBA was found to be the most active compound against the entire gram positive bacterial pathogens tested.[33]

The antimicrobial activity of boswellic acid molecules was studied against oral cavity pathogens. The results showed that AKBA is the most potent antibacterial compound against all the bacteria tested in this experiment.[15] AKBA can be used in the development of antibacterial agent against oral pathogens and can be used in mouthwash for preventing and treating oral infections.[15]

f) Memory

In traditional medicine, Frankincense or olibanum is believed to improve the learning and memory after the consumption and it has been used in elderly for enhancement of memory and in pregnant women to increase the memory and intelligence of their offsprings.[34] The result of a study shows that there is a significant increase in the power of learning at post-learning stage, short-term memory and long-term memory in rats which their mother received aqueous extract of Boswellia serrata orally during the gestational period.[35]

Hippocampus is a sensitive region of the brain involving in certain aspects of learning and memory functions.[36] The dendritic systems are the functional core of neuronal collections as they signify most of the receptive surface of neurons which their organization is essential for integration and transfer of information at the synaptic level.[36] The study indicated that the young rats whose mothers were treated with Boswellia during gestation showed more dendritic branches in CA3 (Cornu Ammonis) pyramidal neurons.[36] In the experiment with prenatally Boswellia treated young rats, the CA3 cells showed obvious expansion of their terminal dendritic arborizations, when compared to the control group.[36] Better learning and memory performance in the offsprings of the mothers who were consuming frankincense during their pregnancy is related to an increase in the somal volume of hippocampal neurons in Cornu Ammonis.[36] These findings suggest that the frankincense and its compounds need to be extensively studied in neurophysiology and possibly for the future treatment of neurodegenerative disorders.[37]

The pharmacological effects of Boswellia seratta was studied by its effect on memory deficits of hypothyroid rats.[34] Many studies have shown that thyroid hormones play a significant role in cell division, maturation, and function of mammalian central nervous system. The deficiency of thyroid hormones in prenatal period can cause growth retardation as well as severe cognitive impairment such as attention, memory processing and general intelligence deficits.[34]

In a study, which hypothyroidism was induced by methimazole in adult male Wistar rats, caused them a significant decline in learning and memory. The use of frankincense has shown to be effective in preventing this deficiency. This result supports the traditional believe that olibanum has beneficial effect in enhancing memory and learning functions.[34]

g) Fertility

Fertility regulation with plant preparation has been reported in traditional medicine and a number of plant species have been tested for their effects on fertility and some of them have been supported by national and international agencies.[9] Frankincense is used by Jordanian population as aphrodisiac and fertility promoting agent. The gum resin of frankincense contains boswellic acids and other pentacyclic triterpenes, which have a chemical structure similar to steroids.[9] In a study which was conducted to examine the effect of Frankincense on the reproductive system

and fertility of adult male rats, the oral administration of Frankincense (Boswellia thurifera) increased fertility in the rats. In addition, the number of implantation and the number of viable fetuses also increased, which may possibly be due to the increase in sperm motility and sperm density.[9] The drug may act on the pituitary gland and increase main hormones of spermatogenesis. Significant increase in sperm motility of cauda edydidymis was observed in treatment group which can be due to the effect of frankincense (B. thurifera) on the enzymes of oxidative phosphorylation.[9]

In conclusion, Frankincense (B. thurifera) resin is a useful compound in fertility mainly by having an effect on pituitary gland cells. More studies are needed to identify the specific method of action of frankincense.[9]

h) Controlled Release of Drugs

Controlled released drug delivery systems are intended to direct the delivery of the drugs to targeted tissues, in desirable and sustaining rates. Among a variety of approaches, preparation of drug-embedded matrix tablets is widely used for this purpose.[38] Although a wide variety of polymers are used in matrix tablets for controlling the drug delivery or improving the bioavailability of the contained drug, the need for safe, natural and effective matrix tablets has always existed.

The use of olibanum resin is considered suitable for the controlled release of diclofenac over 24 hr. (once a day administration).[38] Also in a study on the control release of nifedipine, olibanum and colophony, two natural resins were used as a microencapsulation agents which caused the slow and spread release of the drug over 24 hour.[39]

Olibanum resin is a natural lipophilic polymer which is used as a microencapsulating agent for the good controlled release of the drugs.[40] The result of studies on the matrix tablets formulated with the use of olibanum resin in several drugs like diclofenac, 18 nifedipine, carbamazepine have shown that as the concentration of olibanum resin in the matrix tablets increased the drug release was decreased,[38] which this means the longer stay of drug in the body.[39]

i) Preparation and Dosages

Although different methods of preparations can be formulated for oral, rectal and parenteral administration, the preparations of oral administration are preferred. The pharmaceutical preparations for oral administration may be in the form of tablets or capsules prepared with the use of diluents, such as binding agents, fillers, lubricants, disintegration agents or wetting agents.[28]

The compounds can also be formulated for injection, preferably intravenous, intraarterial, intramuscular, intracranial, intrathecal or subcutaneous and can be in unit dosage form, e.g. in ampoules, or in multiple dose containers with the preservative added. The preparations may be in the form of suspensions, solutions, or emulsions in oily or aqueous carriers.[28]

Boswellia is generally taken orally as a capsule, tablet or its bark decoction. The standardization of Boswellia products is difficult because of variety of Boswellia products.[5] The suggested dosages for inflammatory or asthmatic conditions are 300 to 400 mg of standardized extract (containing 60% boswellic acids) three times daily.[5]

j) Safety

Frankincense, the gum resin of Boswellia, which has been used as a remedy for more than thousands of years has not shown any severe side effects and is considered to be 19 safe.[25] The anti-inflammatory effects of Boswellia unlike of many anti-inflammatory chemical drugs, dose not cause any adverse effects on blood pressure, heart rate, respiration or other autonomic responses with remarkably low toxicity.[17] Gum resin of Boswellia is included in the list of safe substances and its use is permitted by USFDA as a food additive.[15]

Oral preparations of boswellic serrata extract containing AKBA are sold in the market over the counter as anti-inflammatory formulations.[15] The results of many clinical studies have shown that Boswellia is well tolerated in the treatment of rheumatoid arthritis and Crohn's disease with minimum side effects.[29]

Taken together, the side effects of Frankincense, is relatively very low and not severe when compared to modern drugs and their side effects. Then they can be considered quiet safe when are taken in the required and therapeutic dosages.

V. Conclusion

Frankincense has been used in traditional and modern natural medicine for the treatment of variety of illnesses with very minimal side effects. The anti-inflammatory, anti-arthritic, anti-proliferative, anti microbial and analgesic effect of this gum resin can reduce the inflammation and pain in the body and relieve the related symptoms of many diseases. The effect of frankincense is remarkable in increasing the number of dendritic segments and branching in the neuron cells of hippocampus, causing more synaptic connections in that area and therefore improvement of learning and memory. Extensive studies on frankincense and its effect on neurophysiology could be a right approach in finding a possible new complementary or alternative natural medicine to control, cure or 20 prevent some variety of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases.

a) Disclosure Statement

There is no conflict of interest in connection with this manuscript.

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Drug-Induced Disparities in Cell Restoration and Debridement: Ethanol-Triggered Nuclear Misreading of the Restitution Cues

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Abstract- The consequences of the drug-triggered cues from nucleus to cytosol in the restoration of intracellular organelles, cell structure, and function were investigated. The synthesis of the ER-assembled transporters in the cytosol, primed by incubation with nuclei in the presence of ethanol, was manifested by a 26% decrease in the assembly of cell organelle-restoring vesicles, 30% decline in the transport of basolateral cargo, and a 34% amplification in production of the vesicles retained by endosomes. The disparity in the assembly of the Golgi organelle-, cell membrane-, and endosome-directed transporters was in the concurrence with the weakened potential of Golgi-membrane glycosyltransferases and sphingomyelin synthase, amplified acidic sphingomyelinase, and the increased apoptosis. Inadvertently, the composition of the vesicular cohort arriving to Golgi was dictated by ethanol-induced cues released from nuclei to the cytosol that from the outset produced in ER the core assemblies unlike controls.

Keywords: homeostatic cell cycle, disparity in cell restitution, ethanol-triggered decline in Golgi restoration, cell organelle renewal, imbalance in organelle repair, cell debridement.

GJMR-B Classification : NLMC Code: QV 738

Strictly as per the compliance and regulations of:

Drug-Induced Disparities in Cell Restoration and Debridement: Ethanol-Triggered Nuclear Misreading of the Restitution Cues

Amalia Slomiany [α] & Bronislaw L. Slomiany [σ]

Abstract- The consequences of the drug-triggered cues from nucleus to cytosol in the restoration of intracellular organelles, cell structure, and function were investigated. The synthesis of the ER-assembled transporters in the cytosol, primed by incubation with nuclei in the presence of ethanol, was manifested by a 26% decrease in the assembly of cell organelle-restoring vesicles, 30% decline in the transport of basolateral cargo, and a 34% amplification in production of the vesicles retained by endosomes. The disparity in the assembly of the Golgi organelle-, cell membrane-, and endosome-directed transporters was in the concurrence with the weakened potential of Golgi-membrane glycosyltransferases and sphingomyelin synthase, amplified acidic sphingomyelinase, and the increased apoptosis. Inadvertently, the composition of the vesicular cohort arriving to Golgi was dictated by ethanol-induced cues released from nuclei to the cytosol that from the outset produced in ER the core assemblies unlike controls. The evidence presented here provides further support to our concept that multi componential ethanol toxicity is propagated by a disparity in the restoration of the cell organelles, commenced by ethanol-induced erroneous nuclear cues released to the cytosol and reflected in ER synthetic disequilibrium in assembly of the vesicles for the restoration of cell organelles and the cells membranes.

Keywords: homeostatic cell cycle, disparity in cell restitution, ethanol-triggered decline in Golgi restoration, cell organelle renewal, imbalance in organelle repair, cell debridement.

I. Introduction

After organ developmental growth is completed, the restitution program remains and determines the preservation and maintenance of the cells structures and their optimal function in the organ. The fidelity of the renewal relies on a fine tuning of the renovation processes with removal of the resected components by the catabolic debridement [1]-[4]. The intricacy of the seemingly simple, orderly, and methodical process resides in a control of the counteractive biosynthetic processes that fuel the restorative effects [1] [2] [5]-[7]. The activities involve the coordinated synthetic means that power renewal of the cellular organelles and cell membrane by genesis of the fragments rebuilding specific organelles, including those responsible for catabolic cleansing of the cell [1]-[3]. Therefore, the opposing in function actions, intimately connected at the stage where specific sets of mRNA undergoing translocation and translation, concomitant with vesicular membrane synthesis, determine intercalation of the protein into proper lipid arrangements and that both elements are compatible with the structure of the organelle undergoing refurbishing [1] [2] [8] [9]-[11]. Distinctly, the restitution necessitates attuned release of the ER-generated transport vesicles destined to maintain and uphold structural and functional aspects of the cell, including structural and digestive features of the restitution program [11]-[14]. Henceforth, the departure from the norm, embodied in alteration of the homeostatic stable equilibrium predetermining quantities of transport vesicles destined to Golgi and/or mitochondria, may be manifested in pathological outcomes exhibited in a variety of medically recognized complications [15]-[19].

In our studies, the experimentally-induced deviation from the homeostatic equilibrium in the production of Golgi-directed transport vesicles was observed while employing ER with the outer leaflet depleted of serine palmitoyltransferase (SPT) activity [2].The elimination of the outer leaflet SPT (OL SPT) curtailed synthesis of endosome/lysosome-directed transport vesicles without noticeable impact on Golgi-directed vesicular transport responsible for the restitution of Golgi, cellular membrane, and apical and/or basolateral secretion [2] [19]-[21]. Thus, likelihood opened that the agents known to impact the ER to Golgi transport may affect assembly of the diverse sets of Golgi-directed vesicles and generate cellular imbalance by suppressing or promoting Golgi restitution, secretion, cell membrane turnover and lysosomal degradation, or fostering cell restoration and accumulation of undigested autophagosomes [20]-[26]. The demarcation of the outer- (OL) and inner (IL) leaflet SPT activity, by tracking the assembly of the vesicles explicitly minted for their specific contribution in the renewal of the organelles responsible for the cell debridement (OL SPT) from those involved in the Golgi and cell membrane restitution (IL SPT), led us to a thought that disturbed balance in membrane replacements may also affect Golgi's constitutional

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turnover and/or repair [2] [24]-[26]. Thus far, the vesicles-driven restitution of Golgi organelle was not viewed as the separate process that determines the physical fitness of the site that transforms ER-derived vesicles into cell organelles-specific entities. With these features in mind, ethanol, the toxic agents commonly known to inhibit intracellular transport, was investigated by evaluating its effect on the synthesis of Golgi-directed vesicles carrying SM synthase (SMS) and glycosyltranferases (GLTs), the enzymes that reflect restitution of Golgi membrane and determine potential of Golgi to generate sphingomyelin (SM) and an array of the sphingolipids (SphLs) and glycoproteins (GLPs) that govern the cell membrane-specific functions [27]-[31].

Taking into account the findings on the specificity of the vesicular structures rebuilding cell membrane and organellar membranes, we have scrutinized further the impact of ethanol on the aforementioned processes. Its toxicity, as reflected in medically characterized consequences, was tracked from the prime action on the nucleus-initiated processes to its outcomes reflected in formation and delivery of Golgi- and endosome/lysosome- restitution vesicles. Consequently, the principal impact on the terminal apical and basolateral transport was determined by tracing the vesicles containing secretory GLPs and membrane glycosphingolipids (GSphLs) and the vesicles reacting with endosomes. Decline in Golgi-guided transport and the decrease and incomplete glycosylation strongly suggested the attenuation of Golgi restitution and hence impediment of Golgi enzymatic potential to modify vesicular cargo destined to cell membrane [9] [32]-[35]. Alike, the drug-induced inadequate production Golgi restitution vesicles containing SMS implied that inadequate maturation of the basolateral transport vesicles, requiring SM assembly in their membrane, caused inadequate transport to basolateral membrane [30] [31]. Hence, in contrast to the consequences of the depletion of OL SPT [2], the ethanol-induced pathologies were viewed as the results of the nuclear mistiming in the production of the vesicles contributing to the increased potential of the cell catabolic activity which would robustly and viably amplified cellular alterations and apoptotic demise [28]-[31].

As our concept of cell restitution underscores the role of precise tuning of the anabolic and catabolic processes, and the specificity in the vesicles delivery and cell debridement, the evidence presented here provides highly rational and judicious explanation that the large spectrum of ethanol-induced pathologies are initiated by nucleus misguided prompts to refurbish cell organelles and their function. The consequences of balance-derailing drugs in the vitreous experiments allow us to speculate that *in situ* the multitude of cell pathologies are initiated by mistiming of cues determining cell restitution program.

II. Materials and Methods

a) Subcellular Fractionation

For the isolation of cell components the method used herein are well probed techniques described in [2] which allowed us to purify cell and its organelles with minimum breakup of their structure, that cell cytosol was not admixed with fragmented organelles and that cell membranes could be separated from intracellular components.

The cells were prepared from rat liver and gastric mucosa as described previously [2] [22] [26] [32]-[35]. The single cell, incubated in MEM for 3 hours with or without radiolabel, were used for preparation of nuclei [22], subcellular organelles, cell cytosol and cellular membranes [1][2] [36]. In the experiments dedicated to the vesicles synthesis in the presence of drug (ethanol), the translation active control cytosol (CC) or cytosol adjusted to 120 mM ethanol (CEt) were subjected to 30 min incubation with nuclei, centrifuged to separate nuclei and then used in formation of ER-derived vesicles. The preparations of the organelles were rinsed with phosphate buffered saline (PBS), or 0.5M NaCl or urea-PBS, in order to remove the associated residual cytosolic proteins and vesicles that otherwise would remain on their membranes and interfere with analysis of the organelle maternal components. The synthesis of phospholipids (PLs), sphingolipids (SphLs) and protein was assessed using radiolabeled [^{3}H] inositol, [^{3}H] arachidonate, [^{14}C] choline, [^{3}H] serine, [^{3}H] palmitate and [^{32}P] ATP [21] [22] [26] [32] [33] [35]. The cells' ER organelle was incubated with vesicles-depleted CC and/or CEt and specific label named above, and the formed products subjected to separation from organelles as described earlier for organelle-derived biosynthetic transport vesicles [1] [2][9] [13]. The vesicles generated in the control, ethanol-treated and nuclei-conditioned CC and CEt, respectively, were subjected to fusion experiments with Golgi. In turn, thus generated radiolabeled Golgi was subjected to incubation aimed to generate Golgi-derived transport vesicles, followed by separation of the medium containing Golgi-modified transitory transport vesicles destined to endosomes, and/or apical or basolateral membranes. Both incubations that were aimed to generate the ER- and the Golgi-derived vesicles employed vesicles free CC or CEt at concentration of 15 mg protein/ml. The incubation mixture was enriched with components described in [1] [2]. In the experiments dedicated to analysis of the maternal organellar lipid composition and determination of the organelle restitution with newly synthesized radiolabeled ER-derived vesicles, the incubation to generate transport vesicles was repeated in the presence of cytosol derived from CHX-treated cells [1] [37].This step was introduced in order to complete and release from the organelle, the transport vesicles

dedicated for restoration of other than ER, Golgi or endosome organelles. In continuation, the first batch of organelle-derived radiolabeled transport vesicles, whose label was carried from ER transport vesicles reacting with Golgi, was subjected to incubation with endosomes, followed by separation of the endosome and the cytosol containing Golgi vesicles not reacting with endosomes.

b) Preparation of the Control and Ethanol modified Cell Cytosol

The viable cells used for preparation of cell cytosol were homogenized and centrifuged as described previously [1] [2]. After final centrifugation at 100,000xg for 1h, the obtained soluble fraction was adjusted to 15-18 mg protein/ml, admixed with an ATP generating system [2] and referred to as active CC. The vesicle-free cytosol was then admixed with 120 mM ethanol or proportional amount of buffer to which freshly isolated nuclei were added. After 30 min incubation with nuclei, the cytosols were recovered and referred to as control cytosol (CC) and ethanol modified cytosol (CEt).

c) Preparation the Organelles and Membranes derived from CC and CEt Cytosol

The cell membranes and subcellular organelle fractions (mitochondria, ER, Golgi) were recovered from the cold or radiolabeled cells as described earlier [1] [2] [13].The mitochondria and lysosomes were purified from 10,000xg spun fraction [38] [39]-[42]. The lysosomes were recovered from the mitochondrial fraction following treatment that afforded swelling of mitochondria [38] [39] [40] and used for incubation with vesicles derived from CC and CEt. The crude endosomes were isolated from post-mitochondrial supernatant [2] [13] and fractionated on sucrose gradient described in [2]. The pure endosomes were recovered from 35-8% interphase.

d) Assay of the Inner and Outer Serine Palmitoyl Transferase (SPT) of ER

The ER isolated from other cell organelles as described previously [2] washed with 50mM Tris -HCl, 25mM KCl, 5mM magnesium acetate, pH 7.5 (TK buffer) to remove sucrose, suspended in the same buffer with or without 2M urea and incubated on ice for 1h [24]. During this treatment the OL SPT was released from ER and recovered in the 15,000xg supernatant. The IL SPT was retained in the urea-treated ER membranes [2] [24]. Thus obtained fractions of ER containing 50-100 μg protein were used for SPT assays detailed in [2]. After establishing SPT activity in the ER membranes and 2 M urea extracts, further characterization of the fraction containing enzymatic activity was performed to establish whether 120 mM ethanol impacted activity of the urea releasable OL SPT or ER retained IN SPT.

e) Purification of the ER-Derived Vesicles Generated in the Presence of Control or Ethanol-Modified Cytosol

ER- and Golgi-derived transport vesicles were generated in the presence of radiolabeled precursors according to procedure described previously[1] [2][13].The ER or Golgi membranes, mixed with CC or CEt, ATP-generating system, UTP, CTP GTP, fatty acyl CoA and water soluble cold or radiolabeled lipids precursors, were incubated for 30min at 37°C, centrifuged over 0.3M sucrose and treated with stripping buffer at 2°C for 15 min followed by centrifugation at 10,000xg for 10min to separate transport vesicles from ER or Golgi membranes. As indicated earlier, the step of generation of the vesicles from specified organelle was repeated in order to complete and separate synthesized or modified transporters from the maternal organelle. Only the first harvest of transport vesicles was used in the continued experiments. For that, the products separated from maternal membranes were recovered from the supernatant that resulted from centrifugation of the mixture at 150,000xg for 1h. The crude fraction of the transport vesicles was suspended in 55% sucrose, overlaid with 55-30% gradient and centrifuged at 150,000xg for 16 h. The purified transport vesicles were recovered from the gradients as reported earlier [1] [2] [13].

f) Fusion of transport vesicles with ER, Golgi, mitochondria, endosomes and lysosomes

One volume of radiolabeled vesicles (1.3-1.5 mg protein/ml) was suspended in one volume of CC or CHX CC (CEt was used only for the synthesis of ER-derived vesicles) (15mg protein/ml), and added to one volume of cold cell organelles (5mg protein/ml). The reaction was allowed to proceed from 0-30 min at 4°C (control) and at 37°C in the presence of ATP regenerating system [2]. After incubation, the respective organelles were recovered by centrifugation through three volumes of 0.5 M sucrose at 3,000 rpm for 5 min. The ER vesicles recovered from the supernatant after incubation with Golgi were purified on 55-30% sucrose gradient and used in fusion experiments with mitochondria and lysosomes [32][38] [41]. In turn, the Golgi fraction recovered from the first cycle of fusion with ER-derived vesicles generated in the presence of CC or CEt was isolated, introduced to medium generating transport vesicles and, the reaction products of the second cycle of vesicles synthesis, subjected to separation into Golgi maternal organelles and Golgi-derived vesicles. The Golgi-derived vesicles were than incubated with cold endosomes [39]. One volume of the recovered vesicles (0.9-1.1 mg/ml) was suspended in one volume of CC (15 mg/ml) and added to one volume of purified endosomes (5mg/ml). As described for the ER vesicles fusion with Golgi, each reaction was allowed to proceed for up to 30 min and under the same conditions. Finally, the endosomes and the Golgi-

derived vesicles remaining in CC were recovered and their radiolabeled SM and SphL quantitated. At that point, the radiolabeled endosomes and the nonreactive with endosomes Golgi vesicles were subjected to sphingomyelinase (SMase) treatment followed by radiolabeled lipid analysis [1] [2] [43] [44]. In each experiment the fusion of transport vesicles with acceptor organelle was followed by treatment with 2 M urea at 4°C or 0.5M NaCl in order to remove the vesicles which have not undergone *en bloc* fusion. The associated but not fused vesicles were combined with the free vesicles that remained in CC medium. Then, the recovered organelles were centrifuged through 0.5 M sucrose, washed and subjected to radiolabeled lipid analysis. The lipid analyses concentrated on identification of SM, SphL and Cer, the products of Golgi-specific SMS and SM ase degradation, respectively. Therefore, to show SM and Ceramides (Cer), the alkali-susceptible phosphoglycerides (PhGs) were eliminated by subjecting lipid extracts to alkaline methanolysis and then thin layer chromatography along with standards of SM and Cer. In experiments containing radiolabel derived from palmitate labeling, the alkaline degradation products were subjected to one dimensional thin layer chromatography but in two solvent system, first to separate Cer and free fatty acids using mixture of petroleum ethers/ ethyl ethers/acetic acid (80:30:1, v/v/v), while the second solvent system consisting of chloroform/ methanol/ water (65:35:8, v/v/v) was used to identify and quantitate SM that after first chromatography remained at the origin.

g) Apoptosis assay

Quantitative measurement of hepatocytes apoptosis was performed using sandwich enzyme immunoassay (Boehringer Mannheim) directed against cytoplasmic histone- associated DNA fragments [45]. Aliquots of supernatants containing cytosol derived from hepatocytes isolated from controls and alcohol-fed animals [35] or control cytosol and the named subcellular fraction isolated from experiments performed in the presence of CEt was incubated with freshly prepared nuclei, centrifuged to remove the nuclei, and reacted for 90 min at room temperature in the microtiter wells with immobilized antihistone antibodies [45]. After washing, the retained complex was reacted with anti-DNA peroxidase and the immunocomplex-bound peroxidase incubated for 30 min on the plate shaker with 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate reagent for spectrophotometric reading. The values were expressed in apoptotic units per milligram of protein (absorbance at 405nm/mg protein x100). All measurements were conducted on triplicate aliquots derived from each sample.

III. Results

Cell nucleus processes are induced by numerous agents that are capable to impact cell membrane receptors or can penetrate the membrane [10] [46]. Ethanol, one of the later, is readily distributed throughout the body, easily crosses biological membranes and thus instantaneously affects nucleus and virtually all organs and cellular processes [23] [27][29] [32] [47]-[49]. Consequently, its toxicity is linked to the occurrence of many pathological conditions but so far no single mechanism has sufficed to account for the development of a variety of medical problems [18] [23] [47] [48]. Our initial study on ethanol-induced changes in cell function concentrated on ER specific role as the internal base for generation secretory cargo transporting vesicles where the system's synthetic ability was assessed following 30min at 37°C incubation with the drug. These studies demonstrated that the total impact of ethanol was manifested in the 26.4% decline (10,978 vs. 8,080cpm/100 μg protein) in generation of palmitate and serine-labeled ER transporters. Similar trend in ethanol induced cellular performance was determined by quantitation of the secretory glycoproteins assembled in chronic alcohol feeding study which also documented that the posttranslational glycosylation of the secretory cargo decreased drastically [35]. At that stage of our investigations we have not considered the fact that ER-derived vesicles represent precise constructs rehabilitating different cell organelles and that the demand for their synthesis may differ depending on cell derivation, its metabolic status or primary changes that might be induced by the administered drug [1] [2] [9]-[11] [20] [35] [44]. Now, in the context of the investigations that identified spectrum of an organelle-specific ER-initiated transporters [9] [13], the subject confronted here was whether drug such as ethanol affected all, or just the specific group of ER-derived transport vesicles. Particularly, our efforts concentrated on the determination whether drug evoked changes in Golgi-destined products implicated the restitution of Golgi, endosomes, or the cell membrane.

While the widely held view of the acute and the chronic consequences of exposure to ethanol is based on the collective end image of the response to the drug, in this study we have concentrated on ethanol-induced nucleus-initiated processes that impact composition of the cytosol, and henceforth define the consequential events responsible for the production of aforementioned groups of the vesicles. As our concept of cell restitution underscores the significance of nucleus-controlled, balanced responses in the production of the precise quantity and built of the vesicular constructs for every organelle and cell membrane, we put to test an idea that multi componential ethanol toxicity must be initiated in nuclear processes and the aberrant cues released to the

cytosol distort restoration of cell organelles and that culminates in an abnormal cell functions.

To single out and characterize the nucleus initiated cascade of the processes triggered by exposure to ethanol and linked to the synthesis of ER transport vesicles, the cytosol used in assembly of the transporters was admixed with 120 mM ethanol and incubated with freshly isolated nuclei. Thus induced nuclear processes and hence possible alteration of cytosol distinct specificity were measured by the labeling and quantifying the transport products generated in the aforementioned cytosols. Depending on the radiolabeled tracer, (P-choline, or serine), the gradient profiles of the ER-derived vesicles either showed decrease in the total production or were identical with the control (Figure 1). With serine tracer, the labeling of newly synthesized vesicles carrying protein was found to be reduced by 30% (7,224 µg protein harvested from CC-, and 5025µg from CEt-harvested samples), but the serine labeling of the vesicles membrane SPhL core, the Cer, was almost identical. The total label incorporated into the lipids was 19,881 cpm/mg in CC- and 19,757 cpm/mg in CEth-derived vesicles. The lipid label incorporation and the gradient profiles of Golgi-directed transporters suggested that ethanol impacted assembly of the vesicles toan unequal degree, and that was manifested by disproportion in the labeling of the cargo, PhGs and SphLs.

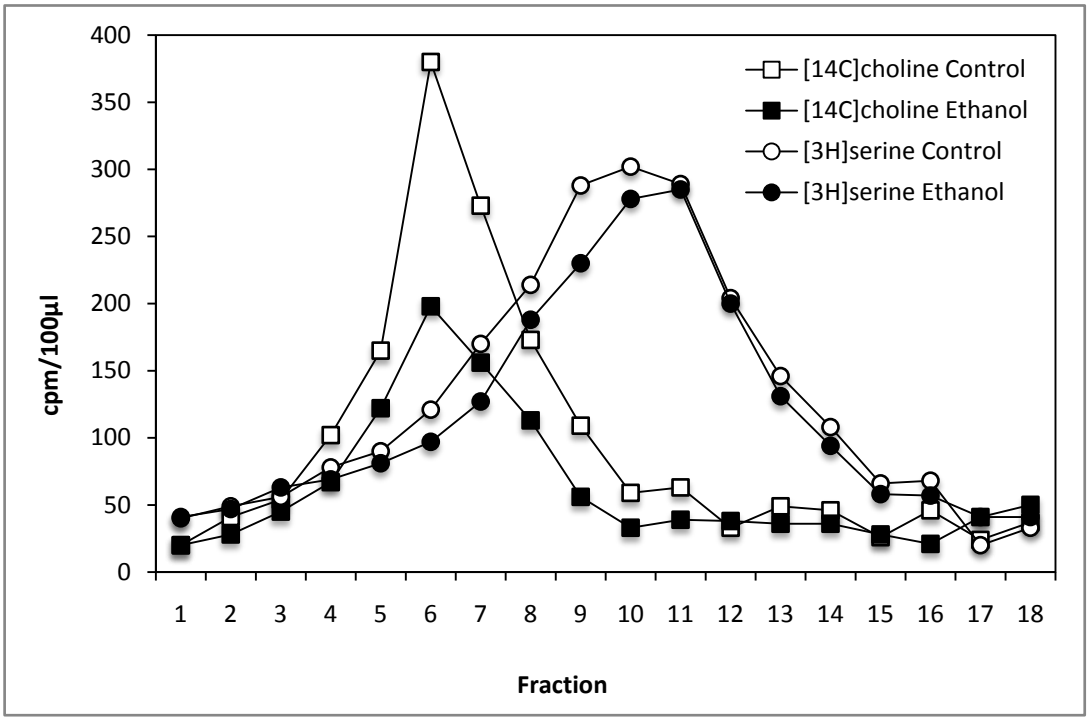
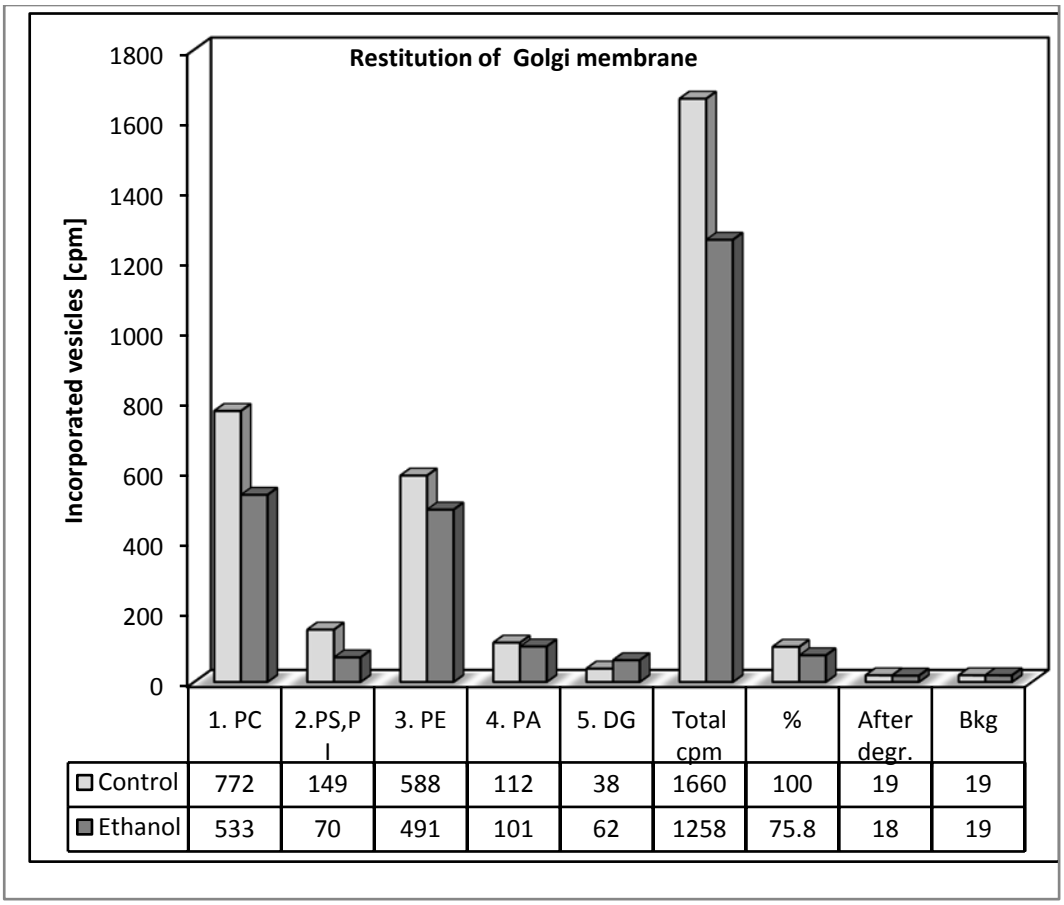


Figure 1 : Quantitative and compositional differences in transport vesicles captured by employing labels identifying P-choline in PLs and serine incorporating into SphLs.

While the entire array of P-choline labeled ER vesicles showed significant reduction in the assembly of the transporters, without P-choline labeled PLs the difference between control- and ethanol-derived ER vesicles was less apparent. Since Cer synthesis was not affected and OL SPT activity was not influenced by its membrane attachment or detachment status [2], we could safely deduce that ethanol treatment was not affecting synthesis of Cer containing ER-derived vesicles destined for endosomes [2]. Rather, with the evidence obtained from earlier investigations of ER-assembled transport vesicles that demonstrated formation of organelle-specific constructs [13], possibility arisen that the spectrum of the ER assembled transporters programmed for apical or basolateral secretion, or restitution of other yet not characterized intracellular organelles, was assembled in modified and the inharmonious quantity. To affirm such likelihood the investigations were carried to determine the relative quantity of the Golgi-, endosome-, and cell membrane-restitution vesicles. The lipids of Golgi membranes labeled by prior incubation with ER-derived vesicles and subjected to consecutive incubation to generate Golgi-derived transport vesicles and then, while depleted of their transitory cargo, evaluated in terms of lipids (derived from ER transport vesicles that permanently incorporated in their membrane as Golgi restitution vesicles)are shown in Figure 2.



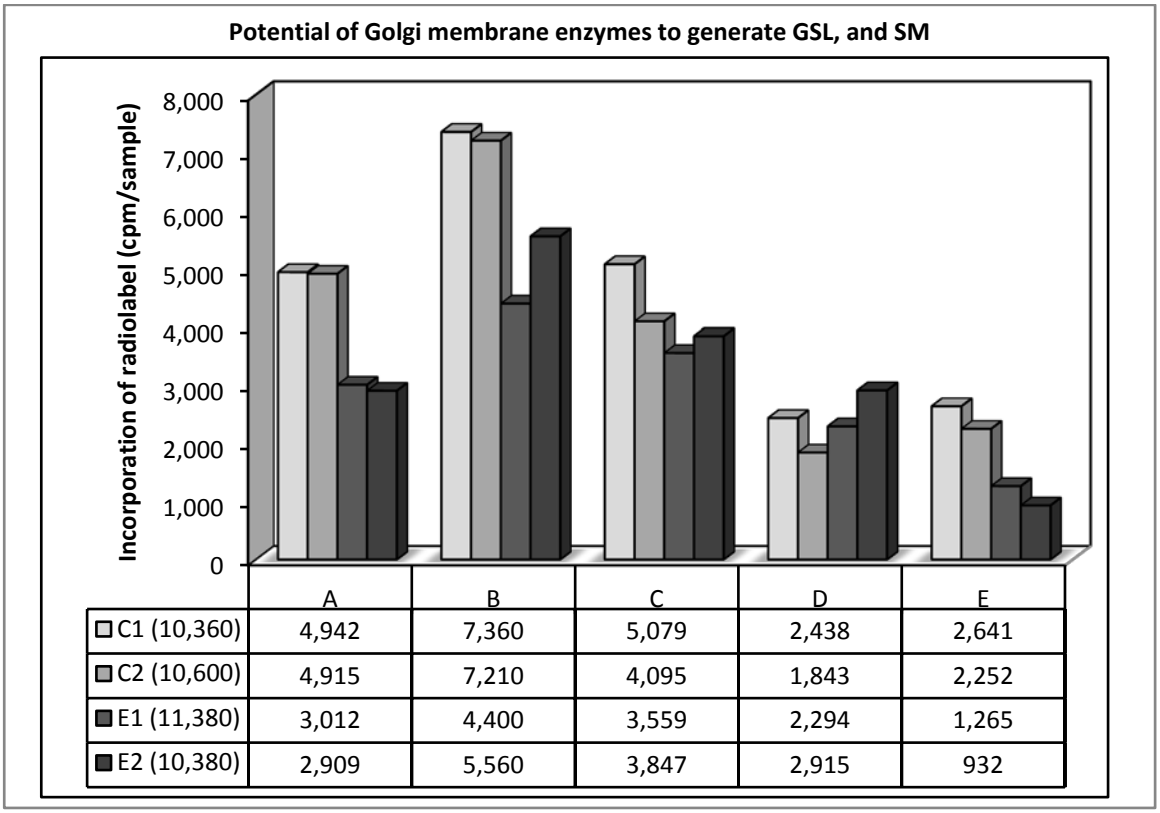
	1. PC	2.PS,PI	3. PE	4. PA	5. DG	Total cpm	%	After degr.	Bkg
Control	772	149	588	112	38	1660	100	19	19
Ethanol	533	70	491	101	62	1258	75.8	18	19

Figure 2 : Quantitative and qualitative differences in restitution of Golgi organelle. The analysis of the labeled lipids that incorporated into Golgi membrane following incubation with ER-derived transport vesicles generated in the presence of unmodified and ethanol-stimulated cytosol. As demonstrated in the analyzed samples, the vesicles incorporating into Golgi membrane constitute of PhGs and glycerides represented by phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA) and diglyceride (DG) without contribution of SphLs.

The analysis of Golgi-derived maternal membranes revealed that Golgi organelle and its restitution vesicles are free of SphLs, and the quantity of PhG-labeled constructs delivered via Golgi restitution vesicles in ethanol derived preparation were reduced by 24.2%. Also, regardless of the sample derivation, (CC or CEt-induced), the Golgi maternal membranes, isolated after repeated incubation and release of the transitory vesicles, were devoid of SphLs and after alkaline degradation of membrane glycerides (**Figure 2**) neither SM, GSL or Cer were found. These results confirmed that reduced radiolabeling of thePhGs in ER-generated transport vesicles reflects diminished production of the vesicles restoring Golgi organelle.

As the potential of Golgi to modify the cargo of the transiting vesicles depends on restitution of the organelle membrane-embedded GLTs and SMS, we have investigated the impact of the reduced rebuilding of Golgi membranes on their activity. By introducing radiolabeled sulfate and comparing its use by the samples of Golgi subjected to incubation with ER transport vesicles carrying equal amount of Cer substrate in CC- and CEt-derived vesicles (**Figure 3, column A**), and by overall production of the SphLs which included synthesis of SM (**column B**), the results showed reduced production of GSLs and SM in the CEth-derived samples. That implied that ethanol-derailed Golgi membranes restitution affected the critical processing of the vesicles destined for cell membrane which was reflected in the drastic reduction in the synthesis of GSphL sulfate, and overall synthesis of SPhLs including SM (**Figure 3, column A, B, respectively**). Thus, based on the lipid compositional analysis of Golgi, and the overall decrease in the vesicles refurbishing Golgi potential to transform ER-derived Cer into GSLs and SM, we concluded that the quantifiable incongruity in ethanol-induced labeling of the Cer-containing ER transport vesicles reflects the fraction representing other than Golgi restitution vesicles. In order to determine which group of Golgi transiting restitution vesicles was affected, our further investigations concentrated on the analysis of the Golgi-

derived Cer-labeled vesicles displaying affinity with endosomes. As illustrated in Figure 3, the amount of Cer-labeled Golgi vesicles was reduced in the ethanol-derived preparations (column C), yet the amount of those with the affinity toward endosomes was higher or equal to that of control (column D). Moreover, the recovery of the vesicles not reacting with endosomes was drastically reduced (column E).



	A	B	C	D	E
C1 (10,360)	4,942	7,360	5,079	2,438	2,641
C2 (10,600)	4,915	7,210	4,095	1,843	2,252
E1 (11,380)	3,012	4,400	3,559	2,294	1,265
E2 (10,380)	2,909	5,560	3,847	2,915	932

Figure 3 : The synthetic activities of the Golgi-specific enzymes utilizing Cer-containing substrate delivered from ER to Golgi through vesicular transport. A-synthesis of GSphL sulphate, B-synthesis of GSphLs and SM, C- the total labeled SM detected in Golgi-generated transport vesicles, D-the labeled SM retained with endosomes following incubation with Golgi-derived vesicles, E- labeled SM in the Golgi-derived transport vesicles not reacting with endosomes. The demonstrated results depict two control- (C1, C2), and two ethanol-(E1, E2) derived preparations, each containing shown amount (in parenthesis) of the labeled Cer.

Thus, compositional analysis of the Golgi-derived Cer-labeled transport vesicles confirmed our initial statement regarding OL SPT activity [2], and showed that the majority of Cer-derived lipids represent endosome-directed transport, whereas the cell membrane directed cargo-carrying transporters are greatly reduced. Using the same amount of Cer labeled Golgi-derived transport vesicles in the reaction with endosomes, we have found that on the average, 34.1 % more of ethanol-derived Golgi vesicles was retained by the endosomes than from the parallel controls (Figure 4).

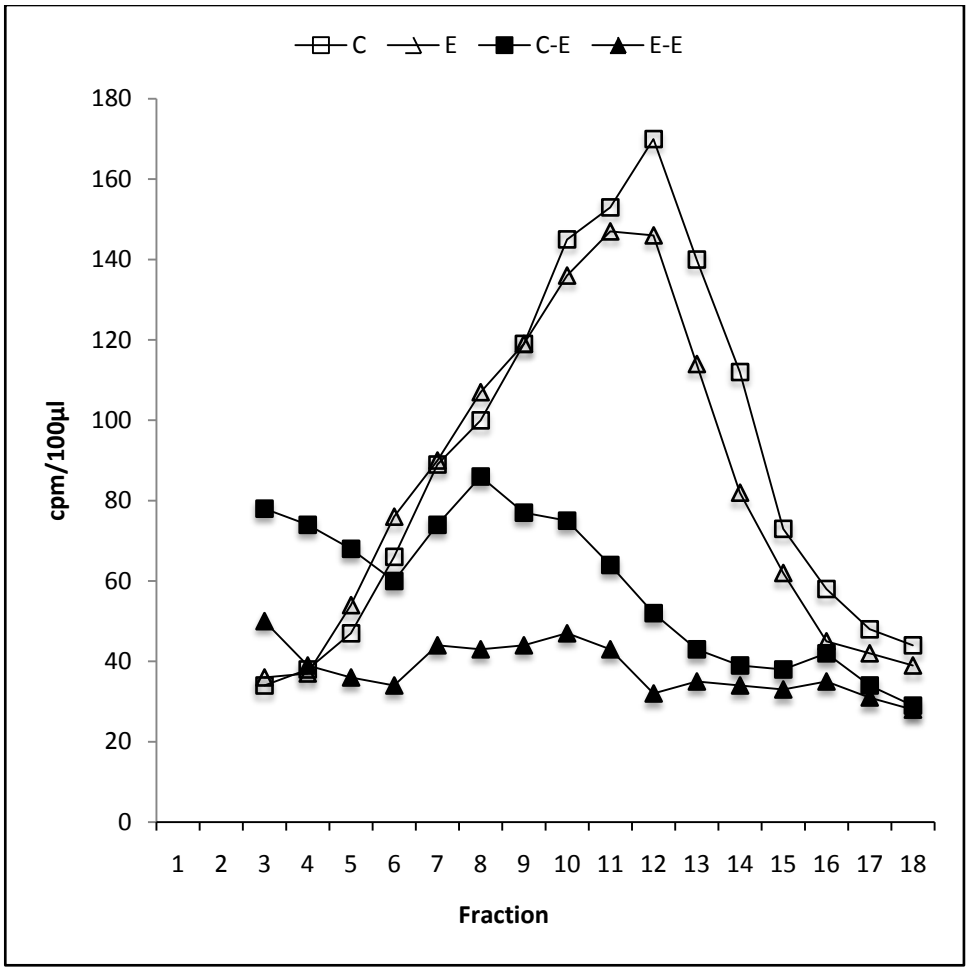


Figure 4 : Golgi-derived transport vesicles reactivity with endosomes. The samples of equally Cer-labeled vesicles from C (C) and CEt (E)before incubation with endosomes (open squares and triangles, respectively) and the fractions remaining free in the cytosol after incubation (closed squares and triangles),were subjected to the sucrose gradient centrifugation. As visualized, the fraction recovered from E samples (EE) was substantially smaller than one from corresponding control (CE). On the average, the CEt-derived preparation (E) consisted of 34% more of the endosome reactive vesicles than the matching control (C).

Thus, this experimental approach gave us strong supportive evidence that the Cer substrate provided in ER-assembled and Golgi-destined vesicles reflected disproportional assembly of the vesicles refurbishing endosome/lysosome organelles andgave us base to speculate that the surplus of Cer-containing vesicles reflects increased production of the endosome directed cargo. Moreover, it confirmed that the Cer is the product of OL SPT, that supplies SM to the outer leaflet of endosome/lysosome membrane. All together, the results suggest that the apparent discrepancy in the ER derived transport vesicles labeled with P-choline showed overall decrease in formation of the vesicles including those responsible for restoration of Golgi. On the other hand, the Cer labeling, as shown in **Figure 1**, demonstrated increased proportion of endosome-directed vesicles which were identified after their release from Golgi and reactivity with endosomes shown in **Figure 4.**

As demonstrated in previous investigations the assembly of the vesicles marked by the SM and GSphLs, is controlled by the provision of Cer on the inner vesicular membrane, and is not affected by the elimination of the ER's OL SPT with which the restitution of endosomes and transport of hydrolytic enzymes have been drastically reduced [2]. In context of these consequences, the results obtained here reveal the drug- induced contrasting outcome; ethanol affects the processes that control translation of the specific mRNA composites aligned with synthesis of SphL controlled by IL SPT. Consequently, the diminished production of the transport vesicles destined to cell membrane debilitates restitution of the cell specific membrane.

While matching amount of Cer incorporated label determined increased quantity of the vesicles with affinity to endosomes (**Figure 4),** further investigations were carried out to determine whether the vesicles carried endosome-specific cargo. The comparison

study between Golgi vesicles delivered SMase contribution to degrade SM substrate by control-derived endosomes with those derived from ethanol-primed samples are depictedin Figure 5. The results depicting the activity of the acidic SMase clearly demonstrate that the ethanol-induced system produced endosome/ lysosomes with higher concentration of hydrolytic enzymes. On the average, the lysosomal SMase potential to degrade SM (EGv minus EC) was 36.4% higher in the CEth-derived samples than those generated by CC.

The outcome of the increased concentration of the catabolic enzymes evoked by the apparent increase in growth of endosome/lysosome organelles was investigated further by performing comparison test of apoptosis induced by ethanol-derived and control preparations. As illustrated in Figure 6, the potential of ethanol-derived samples to invoke apoptosis was 3 fold greater than that of controls.

As demonstrated in Figures 5 and 6, the ethanol-induced Golgi-derived vesicles reactivity with endosomes, the acidic SMase activity, and the degree of apoptosis correlate with the disproportional ethanol-induced restitution of endosomes.

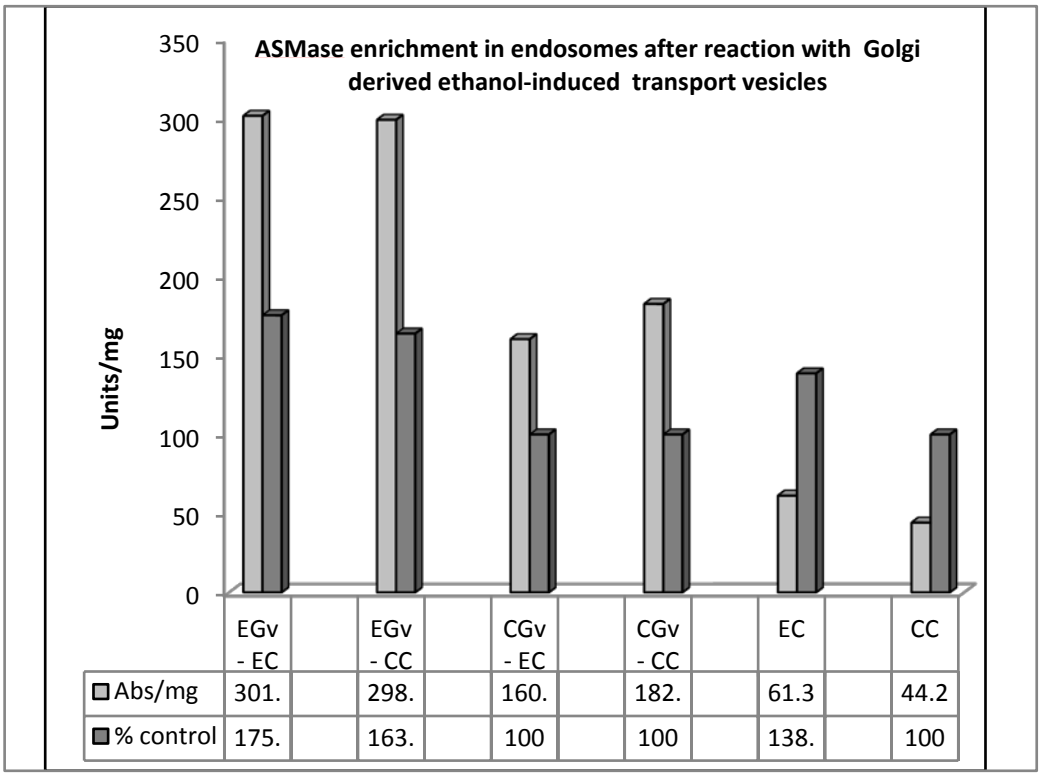


Figure 5 : Comparison of the lipolytic activity of the lysosomal Acidic SMase (ASMase) released from organelles following reaction with Golgi-derived vesicles derived from CEt-and CC preparations. The abbreviations represent Ethanol-Golgi-derived vesicles (EGv), Control-Golgi-derived vesicles (CGv), CEt Cytosol (EC), Control-derived Cytosol (CC).

Assay mixture	Volume used (µl)	ELISA Abs/well	ELISA Abs. average	Abs/ 100 µl	Abs/ 100µg	Induced apoptosis (fold)
EGv + EC	15	0.825 0.876 0.790	0.83	5.540	36.2	8.2
EGv + CC	15	0.899 0.684 0.748	0.78	5.18	34.3	7.8
CGv +EC	15	0.597 0.598 0.615	0.60	4.00	22.1	5.0
CGv + CC	15	0.620 0.620 0.609	0.62	4.1	22.7	5.2
EC	70	0.604 0.828 0.631	0.69	0.99	6.1	1.4
CC	70	0.540 0.739 0.455	0.58	0.83	4.4	1.0

Figure 6 : Induction of apoptosis by the lysosome/endosome preparations following reaction with Ethanol Golgi- and Control Golgi-derived vesicles. As shown, the ethanol derived preparations caused 3fold greater apoptotic demise than the control. The abbreviations describing derivation of the samples are the same as in Figure 5.

The tests whether other cell organelles release to cytosol factors that could contribute to increased apoptotic index were unconvincing. While the ethanol-derived cytosol alone seemed to induce apoptosis to slightly higher degree than control (Figure 6, EC vs. CC), in combination with control-derived vesicles its impact was not observed (Figure 6, CGv+EC vs. CGv+CC). As became evident from the diminished restitution of Golgi membranes and reduced potential of the embedded enzymes to utilize Cer, the query regarding seemingly unaffected synthesis of Cer-containing ER vesicles initially transferred to Golgi has been answered through results of the experiments utilizing endosomes reactivity with Golgi derived vesicles. Together with experiments showing the increased activity of lysosome specific acidic SMase, and induction of apoptosis, the information gathered affirmed that discrepancy between ER-initiated protein-, PhG- and Cer- labeling reflected modified production of Golgi restitution vesicles, decline in the vesicles destined for cell membrane renewal, and substantial upsurge in the replenishment of endosomes and their lytic cargo.

Our experimental approach that relies on specific contribution of lipids in building and restitution of cell organelles and its membrane, not only gave tool to differentiate between vesicles directed to various organelles, but also solidified previous findings that mitochondria and Golgi organelle membranes are free of SphLs [13]. Importantly, and highly relevant to the studies presented here, the approach allowed us to determine the ethanol-induced crucial stages that debilitated Golgi restitution, diminished its potential to transform Cer into SM and adequately glycosylate GSphLs and GLPs cargo [35]. Furthermore, since Cer is exclusively generated in ER membrane during assembly of the Golgi destined transporters, the facts imply that ethanol from the outset of the synthesis divergently influenced the production of the vesicles carrying cell membrane-destined cargo and those assembled for endosomes which in an essence created disequilibrium in restoration of cell and its organelles. Collectively, the results presented above provide convincing argument that the consequences of ethanol-induced nucleus-initiated processes reverberate in the erroneous production of the vesicles restoring Golgi, cell membrane and endosome-specific vesicles. Together, the ethanol-induced derailed repair of the cell may lead to a loss of misguidedly modified cells, or proliferation of the cells with altered glycosylation, appearance, and function.

IV. Discussion

The organization of the key cell identity genes into insulated spaces is recognized as a common trait to all mammalian cell types [5][6] [11] [16] [50] [51]. The continuing challenge is to recognize the remaining critical processes which shape cellular distinct features and depend not only on the principles set in nucleus and ER for the synthesis of protein but on the entire conduit that maintains the cell identity scaffold, the cell

function, and the tissue homeostasis [1] [5] [6] [9] [12] [51]-[53]. Our view, founded on the discovery of cells' transport vesicles specificity [1] [2][9] [13] [32] is consistent with the appropriate regulation of protein synthesis (proteostasis), but advances the concept by extension of the encoded cell-specific protein expression and differences to the next level, one that regulates proteostasis by formation of the nucleus-coined ER-assembled functional segments in form of ER transport vesicles that restore, uphold and maintain cell and tissue homeostasis [1] [2] [9] [13]. The mechanisms that underlie the sustainability of cellular differences are beginning to be elucidated mostly by assessing how these processes change with ageing, cancer, or go wrong in various drug-induced diseases that derail the highly regulated process of such physiological equilibrium (homeostasis) [6] [15] [50] [54]-[59]. But, the global analysis of gene expression, mRNA, or terminal effect of the drug on the specific function have not uncovered their contribution to the multiplicity of yet unmarked steps that may shift the equilibrium in favor of other cellular tasks. As a result, the investigation in numerous laboratories, including our earlier studies on ethanol toxicity, restricted to its effect on cell secretory ability have shown only the cumulative impact demonstrated in the secretory decline of gastrointestinal mucous, hepatic protein, transferrin, and intracellular retention [30] [35] [47]-[50] [55].

Yet the initial and decisive factors influencing processes that reflected alcohol impact on the transport to apical and basolateral restitution and secretion remained unknown. The sight into the intermediary spectrum of the drug-induced toxicity on the secretory activity in the unrelated cells created likelihood that the array of consequences ascribed to alcohol-induced pathologies is initiated at the early and pivotal stage in cell response to the presence of the drug and manifested in specific mRNAs translocation and translation [6] [8] [12] [27] [59] [60]. As ethanol easily crosses all biological membranes and thus affects virtually and simultaneously every organ and biological process, we therefore focused on its primary effects on yet unknown nucleus-instructed processes that dictated generation of the ER-assembled vesicular constructs. Specifically, we have aimed at those manifested in the production of ER-generated transport vesicles that fulfill the structural and functional criteria of the organelles, are responsible for the delivery of apical/basolateral secretion, renovation of the cell membrane, and the restitution of Golgi and endosome/lysosome [1] [2] [6] [14] [17]. Consequently, the ability of ethanol to derange transcriptional and translational regulation initiated in nucleus was explored by exposing isolated nuclei to the cell cytosol with the drug, and then using the nuclei-primed and ethanol-altered cytosol in the synthesis of ER transport vesicles. In our reasoning, the impact of ethanol on nuclear processes should be evident in the release of mRNA assemblies provoked by the drug and be reflected in the make-up of the transport vesicles. In turn, the array of the vesicles assembled in ethanol-induced system could then be verified by determining the Golgi potential to provide transiting transporters with the attributes that direct them to the destination site, and form basic structures that precisely fulfill the cell and organelle designated function [1] [2] [9] [11]-[15] [17].

Specifically, we have aimed at Golgi-specific processing of the ER-delivered vesicles containing SphLs core, the Cer, to produce cell membrane-specific GSphLs and SM and SM-containing vesicles that restore endosome/ lysosome system, the tasks accomplished by Golgi membrane-specific enzymes whose potential rests on the supply of Golgi organelle restoring vesicles [19] [34] [35].As demonstrated herein, the samples calibrated according to quantity of radiolabeled Cer, the marker of Golgi delegated vesicles and utilized by Golgi SMs and GLTs, showed significant differences in enzymatic activity of the Golgi rebuilt with vesicles derived from the cytosol primed with nuclei in the presence of ethanol. Having an equal, quantitavely calibrated Cer-containing fractions, the observed discrepancy in the synthesis of SphLs was not caused by the lack of Cer substrate, but rather by the fact that ethanol-derived vesicles failed to replenish matching quantity of Golgi-specific restitution vesicles delivering organelle explicit SphL-generating enzymes. Indeed, we have demonstrated the impact of ethanol on restoration of Golgi membranes through quantitation of ER vesicles-derived lipids retained in Golgi maternal membranes.

With palmitate label we were able to demonstrate that under the impact of ethanol, the ER released vesicles rebuilding Golgi membranes and hence delivering SMS and GLTs were synthesized in lesser quantity than in controls. The quantitative analysis of the Golgi residual label remaining after prolonged incubation and release of transiting vesicles resulted in the demonstration that Golgi residual membranes recovered from controls afforded samples with larger quantity of radiolabeled glycerides incorporated into its organelle membrane than those from ethanol-derived samples. As demonstrated (Figure 2), the quantity of labeled lipids in ethanol-derived preparations was 24% lower than in parallel controls. Together, the quantitative data on labeled ER vesicles incorporation into Golgi membrane and in P-choline labeled ER-vesicles provided strong support to the belief that ethanol abridged Golgi restitution.

Still, the gap remained in the documentation of Cer-containing vesicles carried to Golgi. In spite of almost equal Cer presence in the ER vesicles carried to Golgi, the lipid composition of the Golgi organelle showed lack of any SphLs in the maternal membrane, the quantity of basolateral transport vesicles containing SM decreased, and the Cer-labeled vesicles were not

retained in Golgi. With plausible explanation of Cer-lacking Golgi-rebuilding vesicles based on the evidence regarding lipid profiles of Golgi residual membranes and Golgi autophagosomes, further studies with Cer-labeled ER vesicles were continued to determine whether Golgi-specific SMSs were equally impeded [2] [23]-[26] [34].

As documented in our earlier studies, the products of Golgi SMSs are specific markers of basolateral transport vesicles containing SM in the inner leaflet of the membrane, and the endosomal/lysosomal transport vesicles with SM in the outer leaflet of the membrane [2] [24].Thus, to distinguish between their activities, the Golgi transport vesicles were subjected to incubation with endosomes, and the amount of the radiolabel retained with endosomes and on unreactive vesicles determined. The results indicated that ethanol-derived Golgi vesicles were up to 34% more reactive with endosomes than corresponding controls. Moreover, the SMase activity of the ethanol-derived endosome were on average 50% higher than in controls. Hence, solid support to the argument was gained that while the restitution of Golgi was impeded, the endosome-directed transporters were not affected and their SMase delivered in substantially higher quantity than that for controls.

Thus, the harmful effect of ethanol outspread beyond inconsistent maintenance of Golgi, beyond Golgi capacity to modify transiting vesicles and their secretory cargo dedicated for cell membrane [1] [2] [25][26][35] but into yet unknown site reflected in the excessive building of the degradative potential of endosomes/lysosomes [47] [49]-[52]. Is it passible that such imbalance in generation of degradative potential of the cell and failure to maintain Golgi and cell membrane generate condition to evoke apoptosis [33]-[35] [45]? While we do not have a direct individual factor that would explain that ethanol-induced delivery of the endosome-reactive vesicles stimulates apoptosis, it is certain that disproportional, not homeostatic release of vesicles that restore components of the cell is creating different attributes in the cell than those created in control, and provide potential for the endosomes to increase catabolic lysosomal processing. Also, based on the presented evidence, we could conclude that ethanol action on nucleus alone, as determined by the activity of nuclei-exposed cytosol in the assembly of ER-transport vesicles, impairs controlled contribution of the restitution vesicles rebuilding Golgi and that reverberates in cell membrane's restitution, cell's secretory activity, and the facilitated lytic potential of the endosomes that may culminate in harming cell vital processes.

Our study, could not support conclusions reached by other investigators that ethanol induces protein retention in Golgi [59], yet the fraction destined for basolateral secretion was substantially smaller than the control suggesting that basolateral transport, just as apical transport was diminished. Is it possible that cytosol houses albumin- and Cer- containing undeliverable vesicles? At this stage of the investigation we cannot disprove or accept the findings since our investigation of the possibility that Cer contribute to apoptotic demise of the cell or that such vesicles exist, have not provided evidence to such an end. All the while the documented events point to the ethanol-induced nuclear release of markers to cytosol which induce processes producing disparity in cell restitution.

The findings on the disparity in cell membrane restitution and delivery of the functional attributes to the membrane, however, are supported by an ample of evidence. The earlier studies demonstrated ethanol-induced functional derailments by inadequate glycosylation of GSphLs, GLPs and reduced transport of albumin [31] [34] [35].The arguments on glycome modification are solidly supported by the studies of chronic alcoholism consequences in the development of mucin glycans, multitude of the changes in glycosylated species of blood-contained GLPs and pathological aftereffects reflected in fetal alcohol syndromes [18]-[20] [23] [28] [30] [34] [35] [59]-[61]. Obtained results inadvertently are linked to the diminished potential of Golgi to modify the transiting transporters and their cargo. The further effects of the derailed delivery of the secretory products, potentiated by the modified appearance of the cell membrane containing inadequately glycosylated GSphLs and GLPs, consequently contribute to the major ethanol-induced aberrant activity of the cells that rely on the specific interaction of their glycome arrays. In each case, the feature that dominates changed appearance of the secreted GLPs, or cell membrane GLPs, or cell membrane GSphLs is the decline in glycosylation. The malfunction is not specific for the failure of one, the pivotal GLT, but all those specific for cis- to trans- Golgi membrane residing enzymes [61][62] [63]. It may be argued that some glycosylation stages are affected to higher degree than other, but it should be remembered that sequential processes of glycosylation will be propagated on the account of deficits in generation of suitable substrate, lack of specific glycosidic determinants or specific ganglioside core structure. It is evident that the manifested changes in glycosylation, determined in an *in vitro*, or chronic studies, are not resulting from the lack of suitable monosaccharide substrates which are provided in the same amount in alcohol and control samples, but clearly reflect the diminished potential of Golgi to glycosylate vesicular membrane and the secretory cargo.

Although some studies suggest that basolateraly secreted cargo, including albumin, remains in Golgi [59], the data on the lack of Cer in Golgi argue against retention of Cer-containing basolateral transporters. And, it is abundantly clear that the albumin carrying basolateral transporters are not rejected prior to

fusion with Golgi, since their glycosylated membrane and glycosylated cargo that reflect intra-Golgi modification are partially accomplished [35] [60]. Thus, it is possible that SM-lacking basolateral transporters remain in the cell cytosol and with prolonged alcohol presence their build-up may contribute to the enlargement of the hepatocytes [31] [47] [49] [59] [62] [64]. Certainly, the feature that determines fusion of the transporters with basolateral membrane is somehow connected with presence of SM in their membrane, without which the vesicles miss the mark. process fails. This attribute of basolateral delivery, sets the process apart from one completing apical delivery, which is accomplished in spite of partial glycosylation of the membrane intercalated GSphLs and GLPs. Therefore, it is possible that deficiently restored basolateral membranes, or inadequately glycosylated cell membranes respond differently to their endocytotic turnover, while the deficits in restoration of Golgi may cause ultimate disappearance of the organelle.

While we cannot provide conclusive evidence explaining all the above described effects, our findings allow us to infer that each of these ethanol-induced outcomes, is initiated by disruption of an equilibrated, cell specific release of the vesicles that prevents organellar disrepair, regulates cell membrane activity and thus maintains fidelity of cellular homeostasis. By extricating nuclear processes, reflected in the synthesis of cellular transporters, we were able to gain insights into relationship between transcriptional control of cell identity by the factors released to the cytosol that drive the expression of key cell identity genes reflected in the transport, timely repair of the organelles, and retention of the equilibrium between restitutive and degradative processes. While the onset of ethanol toxicity depicted in this experimental paradigm may initiate changes at three unseparable stages (transport from cytosol to nucleus, intranuclear processes, and transport out of nucleus) it is certain that the nucleocytoplasmic transport modifications lead to disproportion in the synthesis of organelles-specific vesicles.

Taken together, it becomes evident that the initial nuclear response to the presence of alcohol reflected in the synthesis of multiplicity of transport vesicles can be manifested in every part of the cell structure and function, which in turn allows to explain majority, if not the totality, of the ethanol-induced pathologies of various organs. While it is factual that cytosol derived from ethanol-treated hepatocytes increases cell apoptotic activity, that ethanol toxicity in nucleus evokes disproportions in the assembly of ER to Golgi transporters reflected in an excessive growth of endosome/lysosome, the final conclusion that inadequate cell membrane turnover is responsible for increased apoptotic activity still await further investigation. Particularly, in order to make well grounded conclusion, we must also evaluate the ethanol-induced changes in the cytosol as reflected in the mitochondrial vesicles assembly and their contribution to overall outcome of ethanol toxicity [13].

Collectively, the investigative approach utilized in the presented studies allowed us to decipher the following unknowns: (1) Golgi organelle is free of SphLs which suggests that intracellular organelles differ not only in their protein but also in lipid composition. This has been already established in the case of mitochondrial membranes composition [13], (2) the initial impact of ethanol toxicity is translated into decreased restoration of Golgi, manifested in the lessened potential of its enzymes responsible for assembly of cell membrane SphLs and GSLs, and reduced intercalation of the phosphoglycerides-containing Golgi-specific restitution vesicles, (3) increased synthesis of endosome-directed transport vesicles and an excessive growth and enzymatic potential of endosomes/lysosomes, and (4) reduced output of cellular cargo and diminished repair of cell membrane. Thus, in the final outcome, ethanol caused inadequate posttranslational modification of the cargo relying on Golgi-specific processing, catabolic disproportion reflected in hydrolytic activity of ethanol exposed cells, and the cells with inadequately glycosylated GSL and GLPs that impair and modify their functional characteristics. The above described findings imply that in nucleus, ethanol transmits information that is reflected in misreading of restitution cues, thus causing drug-induced disparity in the cell restitution and debridement.

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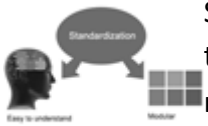

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References

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- Write your paper in the form, which is presented in the guidelines using the template.
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- 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.

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- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

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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.

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	A-B	**C-D**	**E-F**
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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

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