Online ISSN: 2249-4618
Print ISSN: 0975-5888

Global Journal

of Medical Research: B

Pharma, Drug Discovery, Toxicology and Medicine

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VOLUME 14 ISSUE 3 VERSION 1.0

Global Journal of Medical Research: B
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Volume 14 Issue 3 (Ver. 1.0)

Open Association of Research Society

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USA Tax ID: 98-0673427

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Contents of the Volume

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Phytochemical and Pharmacological Evaluation of Fruits of *Sonneratia apetala*

By Anha Afrin Shefa, Farzana Shabnam Baishakhi, Sharmin Islam & Samir Kumar Sadhu
Khulna University, Khulna, Bangladesh

Abstract- *Sonneratia apetala*-Buch.- Ham is a mangrove tree from the family, Lythraceae. The aim of the study is to evaluate analgesic, antidirrhoeal, anthelmintic, cytotoxic activity of the fruits of *S. apetala.* The extract showed dose dependent analgesic activity using acetic acid induced writhing inhibition of the Swiss albino mice. The extract produced 46% and 69% writhing inhibition at the doses of 250 mg/kg and 500 mg/kg body weight respectively while the standard drug (Diclofenac Sodium) produced 82% writhing inhibition at a dose of 25 mg/kg body weight. *In vivo* antidiarrheal activity was substantiated by significant prolongation of latent period and decrease in total number of stools at four dose level as compared to standard loperamide. In anthelmintic test the extract showed significant and dose dependent decrease in paralysis time and death time of *Haemonchus contortus,* where albendazole was used as standard.

Keywords: *Sonneratia apetala*-Buch.-Ham *analgesic activity, antidiarrheal activity, anthelmintic test, brine shrimp lethality bioassay.*

GJMR-B Classification : *NLMC Code: QV 4, QV 17*

Strictly as per the compliance and regulations of:

Phytochemical and Pharmacological Evaluation of Fruits of *Sonneratia apetala*

Anha Afrin Shefa [α], Farzana Shabnam Baishakhi [σ], Sharmin Islam [ρ] & Samir Kumar Sadhu [ω]

Abstract- ***Sonneratia apetala-Buch.-*** Ham is a mangrove tree from the family, Lythraceae. The aim of the study is to evaluate analgesic, antidirrhoeal, anthelmintic, cytotoxic activity of the fruits of *S. apetala.* The extract showed dose dependent analgesic activity using acetic acid induced writhing inhibition of the Swiss albino mice. The extract produced 46% and 69% writhing inhibition at the doses of 250 mg/kg and 500 mg/kg body weight respectively while the standard drug (Diclofenac Sodium) produced 82% writhing inhibition at a dose of 25 mg/kg body weight. *In vivo* antidiarrheal activity was substantiated by significant prolongation of latent period and decrease in total number of stools at four dose level as compared to standard loperamide. In anthelmintic test the extract showed significant and dose dependent decrease in paralysis time and death time of *Haemonchus contortus,* where albendazole was used as standard. In the brine shrimp lethality bioassay, extract showed activity indicated by LC_{50} (61μg/ml) and LC_{90} (616 μg/ml).

Keywords: *Sonneratia apetala*-Buch.-Ham *analgesic activity, antidiarrheal activity, anthelmintic test, brine shrimp lethality bioassay.*

I. Introduction

Plants and herbs have been in use for both cure and prevention of diseases. Man has been experimenting with the plants; some used for food, others for dress, and stills others for treatment of diseases and to keep personnel in a state of health. Researchers are searching for bioactive molecules responsible for specific pharmacological effect in medicinal plants. But edible food can also be a potent source of drug molecules. This leads for the searching of medicinal value of edible fruit. *S. apetala* popularly known as mangrove apple plant belonging to the Lythraceae family and it grows as tree or shrub, distributed through out saline area. The mangrove plant have the anti-HIV, Antibacterial, antiproliferative and antiestrogenic activities and for the treatment of insanity, epilepsy and asthma (Field, 1995). The literature also reports that the leaf part of the plant is widely used for dysentery, sprain & bruises, in treatment of eye troubles (such as cataract) and open sores in children ears and also in heart troubles (Bandaranayake, 1995). The aerial parts of the plant contain a large number of terpenoid, steroid, alkaloid & polysaccharide (Jamale & Joshi, 1998). The antimicrobial activity of the extract of *S. apetala,* on the various test microorganisms, including clinical multiple antibiotic resistant bacteria and phytopathogens were investigated (The Ayurvedic Pharmacopoeia of India.1998 & John, 2007). The seeds contain polyphenols which may be responsible for antibacterial activity and antioxidant activity (Hossain, Basar, Rokeya, Sultana & Rahman, 2012).

Fig.1 : Fruits of *Sonneratia apetala.*

Folk Medicinal Healers in Bagerhat district of Bangladesh uses *S. apetala* as Anti-inflammatory and to treat gastrointestinal disorders (including dysentery, diarrhoea, indigestion, colic, acidity, bloating, lack of appetite, stomachache) (Mollik, Hossan, Rahman,, Rownak & Mohammed Rahmatullah, 2010). There is a number of traditional uses and medicinal benefits that is why this plant part was selected for pharmacological investigations.

II. Materials and Methods

a) Sample Collection

The fruits of *S. apetala* were collected from the world largest mangrove forest-The Sundarbans. Collected fruits were dried by shade drying. Then these were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Author α σ ρ: Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh. e-mail: shefa.pharm@yahoo.com, shefa091127@gmail.com

Author ω: Professor, Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh. e-mail: sadhu1969@yahoo.com

b) Cold Extraction

First extraction- 150gm grinded fruits powder was soaked in 600 ml of ethanol in a glass container for seven days accompanying regular shaking and stirring. After fifteen days the extract was separated from the debris by filtration by a piece of clean, white cotton cloth.

Second extraction- The residue was again soaked in 250 ml of ethanol for three days and then was separated from the debris by filtration through by a piece of clean, white cotton cloth.

Total weight of the dried extract of *S. apetala* was 22gm where percent yield was 14%.

c) Test Animals and Parasites

Young Swiss-albino mice aged 4-5 weeks, average weight 28-35gm were used for the experiment. The mice were purchased from Department of Pharmacy, Jahangirnagar University, Dhaka. They were housed in standard environmental conditions at animal house of Pharmacy Discipline and fed with rodent diet and water ad libitum. All experimental protocols were in compliance with BCSIR Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care. During investigation of anthelmintic property of extract, *Haemonchus contortus* (Nematode), was considered and isolated because it has similarity with parasites living in human body.

d) Phytochemical Screening Methods

For alkaloid analysis the extracts was treated with 1% HCl, boiled and filtered. Dragendorff's was used to indicate the presence of alkaloids (Harborne, 1998) (Trease & Evans 1989), Libermann Burchard and Salkowski tests for the presence steroids, foam test for the presence of saponins, Benedict's and Fehling's test for the presence of carbohydrates, test for the presence of glycoside. 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract indicates the presence of flavonoids. 20gm extract was dissolved in dilute sodium hydroxide and then neutralized with dilute hydrochloric acid. Formation of yellow color and disappearance of color indicate the presence of flavonoid (Agoha, 1981; Barakat, 1973). For the presence of tannins was indicated by ferric chloride and lead acetate test. Presence of gum was evaluated by taking extract and then molish reagent and sulphuric acid were added. Red violet ring was not produced at the junction of two liquids which indicated the absence of gums. To 1 mL of extract, few drops of nitric acid were added by the sides of the test tube and observed for formation of yellow color. This indicates the presence of xanthoprotein. To 0.5 g of the extract, 2 mL of chloroform was added; Conc. H_2SO_4 (3 mL) was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of terpenoids. To the alcoholic extract, sodium bicarbonate solution was added and observed for the production of effervescences. Production of effervescences indicates the presence of acidic compound (Amer, Abou-Shoer , Abdel-Kader, El-Shaibany, Abdel-Salam, 2004)

e) Evaluation of Analgesic Activity

The analgesic activity of extract was studied using acetic acid induced writhing model in mice (Whittle,1964; Ahmed, Selim, Das, Choudhuri, 2004). The animals were divided into control, standard and test groups with five mice in each group. The animals of test groups received test substance at the dose of 250 and 500 mg/kg body weight. Standard group was administered with Diclofenac Na (standard drug) at the dose of 25 mg/kg body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed writhing (constriction of abdomen, turning of trunk and extension of hind legs) for 5 min.

Fig. 2 : Test animal Swiss-albino mice.

f) Evaluation of Antidiarrhoeal Activity

The test was performed by castor oil induced diarrhoea in young Swiss-albino mice (Reiner, R. (1982)). The animals are divided into control, positive control and six test groups containing five mice in each group. Control group receive vehicle (1% Tween-80 in water) at a dose of 10 ml/kg body weight orally. The positive control group receive loperamide (IMOTIL 2 mg/Cap., Square Pharmaceuticals Ltd., Bangladesh) at the dose of 3 mg/kg orally; test groups received the crude extracts at the doses of 250 and 500 mg/kg body weight orally. Each animal was placed in individual cage, the floor of which was lined with blotting paper which was changed every hour. Diarrhoea is induced by oral administration of 0.3 ml castor oil to each mouse, 30 min after the above treatments. During the observation period (4hr), the latency periods and the number of diarrheic faeces excrete by the animals is recorded. A numerical score based on stool consistency is assigned as follows: normal stool =1/2 and watery stool = 1.

g) Evaluation of Anthelmintic Activity

Live parasites *Paramphistomum cervi* (Trematoda) and *Haemonchus contortus* (Nematode) were collected from freshly slaughtered cattle at local abattoirs and identified by Dr Md. Royhan Gofur, Lecturer, Department of Animal Husbandry and Vaterinary Science, Rajshahi University, Rajshahi. During investigation of anthelmintic property of extract, *Haemonchus contortus* (Nematode), was considered and isolated because it has similarity with parasites living in human body. After cleaning, parasites were stored in 0.9% phosphate-buffered saline (PBS) of pH 7.4 prepared with 8.01 g NaCl, 0.20 g KCl, 1.78 g Na_2HPO_4 and 0.27 g KH_2PO_4 in 1L of distilled water at 37±1 ^{o}C. The parasites were divided into different groups consisting of six parasites in each group. Extract at the concentrations of 25, 50, 100 and 200 mg/mL and standard albendazole (info) at the concentrations of 15 mg/mL and 10 mg/mL of 10 mL in PBS were prepared and transferred to petri dishes. Control group was treated with 0.1% tween-80 in PBS. Six parasites were placed in each petri dish and observed. The time of paralysis was recorded when no movement was observed unless shaken vigorously. The death time was recorded after evaluating that the parasites did not move when shaken vigorously, dipped in warm water (50^{o}C) or subjected to external stimuli. Anthelmintic activity was expressed as the time required for paralysis and death of parasites as compared to control.

h) Screening for Cytotoxic Activity

The cytotoxicity assay was performed on brine shrimp nauplii using the method described by Mayer, (1982). Simple zoological organism *Artemia salina* was used as convenient monitor for the screening. DMSO (Dimethyl sulfoxide) solutions of the fractions were applied to *A. salina* in a one-day in vivo assay. For the experiment, 4 mg of methanol and ethyl acetate extracts were dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.123, 1.563, 0.781 μg/ml) were obtained by serial dilution technique. The solutions were then added to the pre-marked vials containing ten live brine shrimp nauplii in 5 mL simulated sea water. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50} and LC_{90}) of the test samples were obtained by plotting percentage of the shrimp killed against the logarithm of the sample concentration and compared with the standard vincristine sulphate.

III. Results and Discussion

a) Phytochemical Screening

The ethanolic extract of *S. apetala* was subjected to qualitative phytochemical tests for detection of different classes of chemical compounds. Alkaloid, reducing sugar, tannin, steroid, glycoside, flavonoids and acidic compounds were present in the extract.

b) Evaluation of Analgesic Activity

At the dose of 250 mg/kg and 500 mg/kg the extract showed inhibition of writhing inhibition by 46.54% and 69.62% respectively while the standard drug Diclofenac Na inhibition was found to be 82.31% at a dose of 25 mg/kg b.w. The result was statistically significant at the level of $p<0.001$ (Table 2). The active principle responsible for this analgesic activity may be acidic compounds, terpenoids, reducing sugar, gums, xanthoprotein, flavonoids and tannins (Ahmadiani, Hosseiny, Semnanian, Javan, Saeedi, Kamalinejad & Saremi. 2000; Rajnarayana, Sripal, Chaluvadi. 2001; Choi, Lee, Park. 2005).

c) Evaluation of Antidiarrhoeal Activity

In the castor oil induced diarrheal mice, extract at various doses lessened the total number of faeces as well as delayed the onset of diarrhea in a dose dependent manner (Table 3). % Inhibition of defecation at doses 62.5mg/kg, 125mg/kg, 250 and 500 mg/kg b. wt. was 74.19, 82.26, 87.90, 94.35 respectively. Standard loperamide also showed decrease in total number of feces and 95.15% inhibition of defecation. It is known that the active component of castor oil is the ricinoleic acid, which is liberated from the action of lipases on castor oil. The ricinoleic acid produces irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins (Yoshio et al., 1999) and stimulating peristaltic (decreasing Na^+ and K^+ absorption) activity and diarrhoea (Zavala, Perez, Vargas & Perez. 1998). Loperamide is a opiate/alkaloid analogue (Tripathi K.D. 2008). It Inhibits prostaglandin synthesis and delay diarrhoea induced with castor oil (Sunil, Bedi, Singla & Johri, 2001). Thus alkaloids of *S. apetala* may follow the same pathway as Loperamide does. Moreover, antidysenteric and antidiarrhoeal properties of medicinal plants were found to be due to alkaloids as well as tannins, saponins, flavonoids, sterols and/or triterpenes and reducing sugars (Havagiray, Ramesh & Sadhna. 2004). These constituents are also present in *S. apetala* responsible for antidiarrhoeal activity.

d) Evaluation Of Anthelmintic Activity

In the present study the *S. apetala* was found to show anthelmintic activity when compared to albendazole used as standard drug. Extract at concentrations of 25, 50, 100 and 200 mg/ml showed paralysis at, 441.5, 276, 255, 209 seconds and death time found at 615, 438, 354, 222 seconds respectively. Standard albendazole also showed paralysis time 334, 273 seconds and death time at 379, 338 seconds for concentration of 10 and 15 mg/ml respectively. From the above result, it is clear that *S. apetala* has significant

anthelmintic activity in dose dependent manner which was comparable with standard anthelmintic drug. This also supports its traditional use as anthelmintic. Albendazole inhibits parasitic microtubule polymerization by binding to β-tubulin (Goodman LS., Gilman A. 11th Edn). As *S. apetala* showed anthelmintic activity compared to albendazole, may be their pathway is same.

e) Screening for Cytotoxic Activity

In brine shrimp lethality bioassay, the crude extract of *S. apetala* fruit showed lethality indicating the biological activity of the extract. The percent of mortality vs log concentration was plotted and a best fit line was obtained using LDP line probit analysis software. Through the software LC_{50} and LC_{90} of the test sample were found to be 61μg/ml and 616 μg/ml. As it contains many tanins and other polyphenols, its cytotoxic effect may be correlated some polyphenols perturb the membrane structure (Hossain 2002; Aoshima, 2005).

IV. Conclusion

The fruit of *S. apetala* is extensively consumed in coastal areas of Bangladesh without any known toxicity in humans. Based on our present observations *S. apetala* has many *in vitro* and *in vivo* biological activity. It may be concluded that this mangrove fruit merits further exploration both chemically and biologically to identify the functional principle(s) and mechanism of action.

V. Acknowledgement

Hereby, the authors would like to thank Pharmacy Discipline, Khulna University, Khulna, Bangladesh for the financial and personnel support during this research.

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Table 1 : Phytochemical Investigation of Ethanolic Extract of *S. apetala* Fruits

Phytochemical Group	Result
Reducing sugars	+
Tannins	+
Flavonoids	+
Saponins	-
Gums	-
Steroids	+
Alkaloids	+
Glycosides	+
Proteins	-
Terpenoids	+
Acidic compounds	+

Table 2 : Effect of *S. apetala* on Acetic Acid Induced Writhing of Mice

Group	Treatment and Dose	Number of writhes (% Writhing)	% Writhing Inhibition
Control	1% tween 80 solution 10ml/kg, p.o.	26±2.003 (100)	---
Standard	Diclofenac Na 25 mg/kg p.o.	4.6±0.65** (17.69)	82.31
Test Group-I	*S. apetala* extract 250 mg/kg p.o.	13.9±1.825** (53.46)	46.54
Test Group-II	*S. apetala* extract 500 mg/kg p.o.	7.9±2.25** (30.38)	69.62

Note: 1. Values are expressed as mean± SEM (Standard Error Mean);
*2. ** indicates P>0.001;*
3. one way ANOVA followed by Dunnet's test as compared to control;
4. n=Number of mice; p.o.: per oral.

Table 3 : Effect of *S. apetala* on Castor oil Induced Dirrhoea in Mice

Group	Dose(/kg-bw)	Mean Latent Period in min ± SEM	Mean Number of Stool in 4hr ± SEM
Control	1% tween-80 in distilled water	36.2±3.54	24.8±1.68
Standard	Loperamide	191.6±3.52	1.2±0.18
Test Group I	*S. apetala* extract 62.5mg p.o.	63.2±3.08	6.4±1.08
Test Group II	*S. apetala* extract 125mg p.o.	77.2±2.82	4.4±0.46
Test Group III	*S. apetala* extract 250mg p.o.	112.6±3.64	3±0.05
Test Group IV	*S. apetala* extract 500mg p.o.	183.4±10.06	1.4±0.36

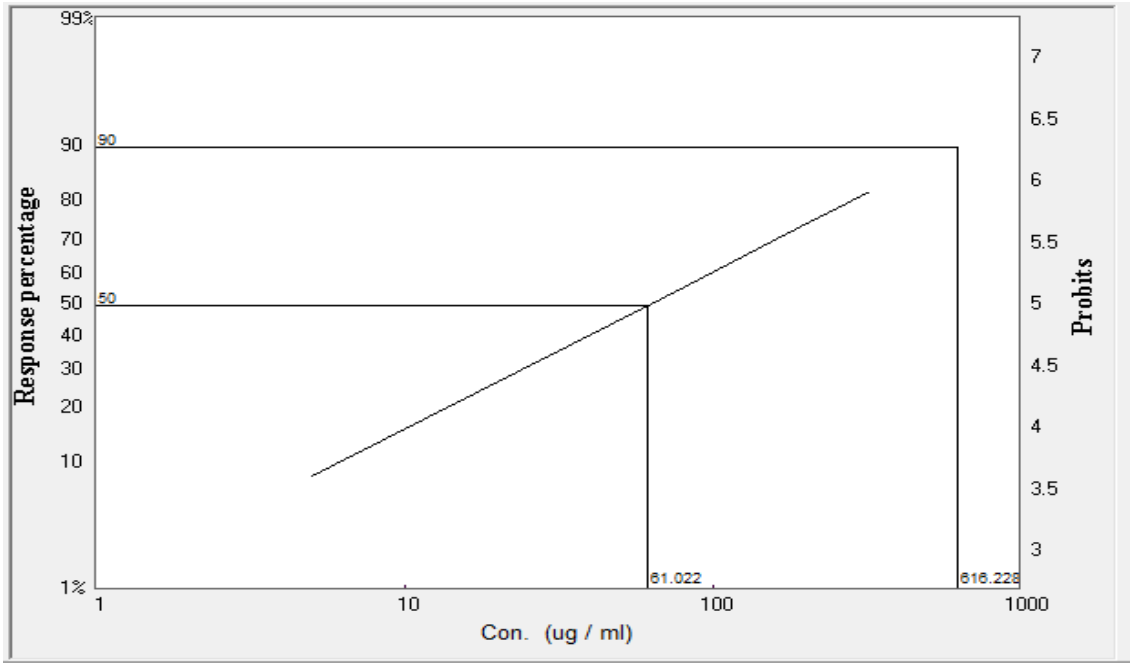
Note: 1. Values are expressed as mean±SEM(Standard Error Mean);
*2. ** indicates P>0.001;*
3. one way ANOVA followed by Dunnet's test as compared to control;
4. n=Number of mice five; p.o.: per ora.

Table 4 : Result of Anthelmintic Activity Test of *S. apetala*

Group	Concentration (mg/ml)	Time in seconds	
		Paralysis	Death
Control	0.2% Tween-80 in water	---	---
Standard Group I	Albendazole 10	334.17±5.45	379.17±6.17
Standard Group II	Albendazole 15	273.33±3.26	338.67±2.42
Test Group I	*S. apetala* extract 25	441.5±7.58	615.67±4.45
Test Group II	*S. apetala* extract 50	276.67±7.78	438±9.70
Test Group III	*S. apetala* extract 100	255±3.76	354.83±7.42
Test Group IV	*S. apetala* extract 200	209±5.11	222.16±4.12

Note: 1. Values are expressed as mean±SEM(Standard Error Mean);
*2. ** indicates P>0.001;*
3. one way ANOVA followed by Dunnet's test as compared to control;
4. n=Number of mice;

Table 5 : Graphical Representation of Brine Shrimp Lethality Bioassay and Both LC_{50} and LC_{90} for the *S. apetala* by Ldp Line Software



Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Acute and Sub-Acute (28-Day) Oral Toxicity Studies of Ethanolic Extract of *Celtis Timorensis* Leaves in Rodents

By Prasanth Kumar. M, Suba. V, Ramireddy. B, & Srinivas Babu. P
JNTU Hyderabad, India

Abstract- Aim of the study: The present study was carried out to evaluate the safety of ethanolic extract of *celtis timorensis* (EECT) by acute and sub-acute toxicity studies.

Materials and Methods: Acute toxicity study was conducted in mice by using OECD 425 guidelines whereas sub-acute toxicity study was carried out in rats by using OECD 407 guidelines. In the acute toxicity study, mice were administered a single dose of 2000 mg/kg and 5000 mg/kg orally and then observed individually for the first four hours, then over a period of 24 hours and at least once daily for 14 days. In the subacute toxicity studies, EECT was given orally at doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight daily for 28 days to male and female rats respectively. General behavior, adverse effects and mortality were observed throughout the experimental period. Food intake, water intake, body weight, organ weight, hematological and biochemical parameters, histopathological changes were evaluated.

Results: The limit doses of 2000 mg/kg and 5000 mg/kg did not cause any mortality or signs of acute toxicity in the mice tested during the observation period. In sub-acute toxicity tests, the results did not show any treatment related abnormalities in terms of hematological and biochemical parameters.

Keywords: celtis timorensis, acute toxicity study, subacute toxicity study, rodents, biochemical parameters and hematological parameters.

GJMR-B Classification : NLMC Code: QV 50

Strictly as per the compliance and regulations of:

Acute and Sub-Acute (28-Day) Oral Toxicity Studies of Ethanolic Extract of *Celtis Timorensis* Leaves in Rodents

Prasanth Kumar. M [α], Suba. V [σ], Ramireddy. B [ρ], & Srinivas Babu. P [ω]

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Results: The limit doses of 2000 mg/kg and 5000 mg/kg did not cause any mortality or signs of acute toxicity in the mice tested during the observation period. In sub-acute toxicity tests, the results did not show any treatment related abnormalities in terms of hematological and biochemical parameters. There were no significant differences in body weight and organ weight between the control and treated groups. No morphological changes were observed in the histopathological analysis of the major vital organs (liver, kidney, stomach, spleen, brain and heart) tested.

Conclusion: These results concluded that the EECT did not cause any mortality and signs of toxicity in mice (acute toxicity study) and rats (sub-acute toxicity study). The oral lethal dose of ethanolic extract is more than 5000 mg/kg and no-observed-adverse-effect level (NOAEL) of the extract for both male and female rats is 1000 mg/kg per day for 28 days.

Keywords: celtis timorensis, acute toxicity study, sub-acute toxicity study, rodents, biochemical parameters and hematological parameters.

Author α: Research scholar, Faculty of Pharmaceutical Sciences, JNTU, Hyderabad, Andhrapradesh.
e-mail: prasanth.pharmacology@gmail.com
Author σ: Assistant professor, Pharmacology, National Institute of Siddha, Tambaram Sanatorium, Chennai.
Author ρ: Director-Formulations, NATCO Pharma, Hyderabad, Andhrapradesh.
Author ω: Principal, Vignan Pharmacy College, Vadlamudi, Guntur, Andhrapradesh.

I. Introduction

Natural products which included herbs, animals and minerals serve as the lead compounds for the development of new medicines and also for the treatment and prevention of various human ailments. The present accepted modern medicine has gradually developed in the recent years by various efforts done by the researchers. However, traditional medicine still remains as basis in the development of new drugs [1]. In recent years, herbs and herbal medicines have continued to receive interest and attention from the people as these products are safe and free from side effects [2]. The growing number of herbal drug users around the globe and lack of scientific data on the safety profile of herbal products make it necessary to conduct toxicity study of herbal products [3]. Toxicity associated with herbal products has alerted many national and international regulatory authorities to develop and implement various set of guidelines for assessing, monitoring and preventing the toxicity associated with the herbal products. For example, Uppsala monitoring committee (UMC) of the world health organization (WHO) collates and communicates information regarding herbal adverse drug reactions whereas Organization for Economic Cooperation and Development (OECD) sets guidelines for conducting various toxicity studies. Toxicity tests are most widely used to examine specific adverse events or specific endpoints such as cancer, cardiotoxicity and skin/eye irritation. Toxicity testing also helpful in determining the No Observed Adverse Effect Level (NOAEL) dose and is helpful for further clinical trials [4]. Acute, sub-acute and chronic toxicity tests are routine toxicity tests carried out by the pharmaceutical companies in the development of new medicines. In order to assess the toxic nature of a compound, acute oral toxicity is the first step to be carried out [5]. Acute toxicity testing involves the determination of lethal dose, the dose that kills 50% of the tested group of animals, whereas sub-acute and chronic toxicity testing involves the determination of long term effects of the test compound upon repeated administration.

celtis timorensis belonging to cannabacea family is a flowering plant commonly known as stinking

wood or stink wood. The plant has been recommended in ayurveda for the improvement of memory and in the treatment of nervous disorders. The plant extract has been reported for antidepressant, anticonvulsive and nervous disorders. The extract also enhanced learning and memory in humans [6]. It also helps in repairing of neurons which were damaged in specific brain regions. The plant extract also showed a neuroprotective effect against oxidative stress in the hippocampus of rat brain [7]. Traditionally the leaf extract of *celtis timorensis* is given during dysentery conditions [8]. Despite the various uses over long time periods, no toxicological data is available regarding the safety of repeated exposure to *celtis timorensis*. As a part of safety evaluation, acute and sub-acute oral dose toxicity studies were carried out to investigate the potential toxicity after single oral dosing of extract in mice and 28-day repeated oral dosing of extract in rats.

II. Materials and Methods

a) Collection and Identification of Plant Materials

Fresh leaves of *celtis timorensis* were collected from Tirupathi, Andhrapradesh. The plant was identified and authenticated taxonomically by Assistant professor K.Madhava chetty of the Department of Botany, S.V. University, Tirupathi, Andhra Pradesh, India. A voucher specimen of the collected sample was deposited in the herbarium of the institution for future reference.

b) Preparation of the Extract

The leaves are shade dried and made into coarse powder and extracted with 70% ethanol by cold maceration method for 72 hours with intermittent shaking. The extract was filtered and concentrated at high vacuum. The extract was stored in the refrigerator till further use.

c) Animals

Swiss albino mice (25-30 g) were selected for acute toxicity studies and Wistar albino rats (weighing between 130 gms-200 gms) of both sexes were selected for sub-acute toxicity studies. They had free access to food and water and were maintained under standard laboratory conditions which included 12-hour light-dark cycle and temperature of 28-30 degrees centigrade. Animals are allowed for a one week of acclimatization period prior to the study. The experimental protocol was approved by the IAEC (institutional animal ethical committee) and care of the experimental animals was taken according to the CPCSEA guidelines.

d) Acute Toxicity Studies

Acute toxicity studies of ethanolic extract of *celtis timorensis* (EECT) was carried out in female mice by using Organization for Economic Co-operation and Development (OECD) guideline 425 [9]. Before oral administration of a single dose of the test samples, the mice were deprived of food for 3 h. Doses of 2000 and 5000 mg/kg of the test samples were given using oral gavage to mice of Group I and Group II respectively. All the mice were observed for general behavioral changes; symptoms of toxicity and mortality after treatment for the first four (critical) hours, then over a period of 24 hours, thereafter daily for 14 days.

e) Sub-Acute Toxicity Studies

Sub-acute toxicity study (28-day repeated oral toxicity study) was carried out according to OECD 407 guidelines [10]. Both sexes of rats (130-200g) were divided into four groups with 10 animals (5 males plus 5 females in each). The group I received 1% CMC vehicle orally at a dose volume of 10 ml/kg body weight and served as a control group whereas group II, group III and group IV received EECT at 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight, p.o. respectively (10ml/kg body weight dissolved in 1% CMC). All the groups of rats were observed twice daily for mortality and morbidity till the completion of the experiment. All the animals were observed for clinical signs and the time of onset, duration of these symptoms, if any were recorded. Body weights of the rats in all groups were recorded once before the start of dosing, once weekly during the treatment period and finally on the day of sacrifice. The amount of food and water intake was recorded on every day and the data were expressed as 7 days cumulative value. At the end of the experiment (on 29th day), blood samples were collected from overnight fasted rats (only water allowed) by retro-orbital bleeding into heparinized and non-heparinized tubes for hematological analysis and biochemical analysis.

f) Hematological parameters

The heparinised blood was used for the analysis of hematological parameters such as hemoglobin, red blood cell count, white blood cell count, platelet count were measured using fully automated hematology analyser (PE 6000).

g) Biochemical Parameters

The serum was separated from non-heparinized blood and the serum biochemical parameters including total cholesterol, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), triglycerides, total cholesterol, albumin, bilirubin and total protein were analysed by using semi-automatic biochemical analyser (Star 21plus, India).

h) Histopathology

After blood collection on day 29, all the animals are euthanized for gross pathological examinations of all major internal organs. Organs such as liver, kidney, stomach, brain, heart and spleen were collected from all the animals for histopathology. The collected organs were weighed and preserved in 10% neutral buffered formalin, trimmed and a 5μ thickness of tissue sections

were stained with hematoxylin and eosin for histopathological study.

i) Statistical Analysis

Results are expressed as mean ±standard error mean (SEM). Data obtained was analyzed by using one way ANOVA followed by Dunnett's test and $p<0.05$ was considered as statistically significant.

III. Results

a) Acute Toxicity Studies

In the toxicity study, oral administration of the EECT at 2000 mg/kg and 5000 mg/kg did not produce any deaths and clinical signs of toxicity in mice. As there were no mortality and clinical signs of toxicity in both the tested doses, LD50 value of EECT was found to be greater than 5000 mg/kg.

b) Sub-Acute Toxicity Studies

There were no treatment related toxicity signs and mortality observed in both sexes of rats treated at 250mg/kg, 500mg/kg and 1000mg/kg orally during the 4 weeks of treatment. No significant differences in body weight were observed between the initial and final body weight of the rats treated with EECT and control rats (Table 1). A similar absence of toxic effect was observed in the case of food and water consumption (Table 2 and Table 3). There were no significant differences between control and EECT treated groups in organ weight (Table 4). The hematological profile of treated and control group were summarized in Table 5. The results concluded that all hematological parameters such as total red blood cell count, total white blood cell count, platelet count, haemoglobin, hematocrit and differential leukocyte count are with in normal range in both control and treated groups during the experimental period. The data on biochemical parameters in treated and control rats were presented in Table 6. Sub-acute administration of EECT did not show any significant changes in biochemical parameters such as creatinine, urea, triglycerides, total cholesterol, total protein, albumin, aspartate aminotransferase (AST), alanine aminotra-nsferase (ALT), alkaline phosphatase (ALP) and total bilirubin when compared to control groups. There were no statistically significant differences in the hematological parameters and biochemical param-eters measured between control and EECT treated groups. In our study, we performed histopath-ological examinations in control and high dose group-brain, heart, liver, kidney, stomach and spleen and they were revealed no abnormalities. Hence we did not performed histopathology examination of low and medium dose groups. The No-Observed Adverse Effect level (NOAEL) of the extract was estimated to be greater than 1000 mg/kg/day in rats. Hence it can be concluded that EECT is safe for oral administration.

Table 1 : Effect of Ethanolic Extract of *Celtis Timorensis* (EECT) on Body Weight Gain in Rats-Sub-Acute Toxicity Study'

Treatment group	Body weight (gms)					
	Sex	Day 1	Day 7	Day 14	Day 21	Day 28
Control (1% CMC)	Males (n=5)	167.00±4.35	173.20±5.17	176.00±5.09	179.00±4.58	182.00±3.74
	Females(n=5)	158.00±9.16	162.20±7.27	164.40±8.35	167.60±6.91	170.60±7.63
250 mg/kg	Males (n=5)	174.00±8.27	180.60±7.40	185.00±7.58	189.00±7.81	193.00±8.45
	Females(n=5)	161.40±9.92	164.60±9.56	168.40±9.75	169.40±9.94	173.80±10.4
500 mg/kg	Males (n=5)	180.00±6.12	186.00±5.56	191.00±5.56	194.00±4.84	199.00±3.31
	Females(n=5)	155.40±9.60	161.20±9.30	164.00±9.48	167.20±11.1	170.80±11.3
1000 mg/kg	Males (n=5)	184.00±5.78	193.00±5.38	197.00±5.61	202.00±5.61	205.00±4.47
	Females(n=5)	162.60±11.7	166.60±10.2	166.80±10.3	170.00±10.8	176.40±11.7

Values are expressed as mean±SEM, n=5 females and 5 males

Table 2 : Effect of Ethanolic Extract of *Celtis Timorensis* (EECT) on Feed Intake in Rats-Sub-Acute Toxicity Study

Treatment group	Gms				
	Sex	First week	Second week	Third week	Fourth week
Control (1% CMC)	Males (n=5)	44.72±1.30	39.11±2.81	42.58±2.2	43.25±1.46
	Females(n=5)	40.15±1.50	40.55±1.75	39.17±2.42	41.45±1.68
250 mg/kg	Males (n=5)	40.87±1.67	36.12±2.24	38.50±1.84	44.85±2.03

	Females(n=5)	40.68±2.32	35.48±2.63	44.07±1.97	40.07±2.49
500 mg/kg	Males (n=5)	39.97±3.03	41.58±3.07	41.30±3.25	41.22±3.41
	Females(n=5)	41.80±2.41	39.87±2.29	38.60±1.79	42.52±2.13
1000 mg/kg	Males (n=5)	39.67±2.03	39.82±1.90	43.50±1.50	44.55±1.60
	Females(n=5)	41.55±2.01	43.44±1.37	47.25±1.90	44.95±1.13

Values are expressed as mean±SEM, n=5 females and 5 males.

Table 3 : Effect of Ethanolic Extract of *Celtis Timorensis* (EECT) on Water Intake in Rats- Sub-Acute Toxicity Study

Treatment group	(ml)				
	Sex	First week	Second week	Third week	Fourth week
Control (1% CMC)	Males (n=5)	55.85±3.33	46.71±2.86	49.71±2.69	58.42±2.67
	Females(n=5)	45.57±2.71	44.92±2.32	48.21±2.21	48.42±3.82
250 mg/kg	Males (n=5)	49.28±2.74	43.28±1.86	57.00±3.65	53.28±2.56
	Females(n=5)	48.00±2.13	46.42±2.09	49.28±3.62	46.85±2.26
500 mg/kg	Males (n=5)	52.57±3.19	56.14±3.54	55.57±3.06	51.57±1.51
	Females(n=5)	44.71±2.37	51.85±2.29	47.85±3.32	52.01±0.95
1000 mg/kg	Males (n=5)	49.85±2.08	55.57±1.41	50.57±3.18	51.57±3.48
	Females(n=5)	48.64±1.78	50.71±2.84	48.21±2.14	47.14±2.36

Values are expressed as mean±SEM, n=5 females and 5 males.

Table 4 : Effect of Ethanolic Extract of *Celtis Timorensis* (EECT) on Body Organ Weight in Rats-Sub-Acute Toxicity Study

Organ weight (gms)	Treatment group							
	Control (1% CMC)		250 mg/kg		500 mg/kg		1000 mg/kg	
	Males n=5)	Females (n=5)	Males (n=5)	Females (n=5)	Males (n=5)	Females (n=5)	Males (n=5)	Females (n=5)
Liver	4.79±0.32	4.58±0.53	5.87±0.32	4.98±0.30	5.93±0.45	5.38±0.39	5.07±0.49	4.71±0.53
Brain	1.52±0.03	1.48±0.04	1.57±0.07	1.55±0.05	1.61±0.06	1.55±0.05	1.53±0.04	1.57±0.06
Spleen	0.43±0.03	0.47±0.02	0.39±0.04	0.44±0.02	0.47±0.02	0.45±0.04	0.48±0.04	0.45±0.03
Kidney	1.07±0.10	1.04±0.08	1.13±0.05	1.17±0.07	1.22±0.08	1.16±0.08	1.17±0.13	1.16±0.06
Heart	0.69±0.01	0.62±0.05	0.72±0.02	0.68±0.02	0.74±0.04	0.65±0.02	0.75±0.03	0.65±0.03
Stomach	2.17±0.14	2.21±0.21	2.43±0.25	2.77±0.25	2.38±0.32	2.42±0.25	2.96±0.29	3.12±0.26

Values are expressed as mean±SEM, n=5 females and 5 males.

Table 5 : Effect of Ethanolic Extract of *Celtis Timorensis* (EECT) on Hematological Parameter In Rats-Sub-Acute Toxicity Study

Hematological Parameter	Sex	Treatment group			
		Control	250 mg/kg	500 mg/kg	1000 mg/kg
Hemoglobin (g/dl)	Males (n=5)	14.74±0.50	14.30±0.75	14.34±0.58	13.72±0.67
	Females(n=5)	15.68±0.59	16.24±0.30	15.76±0.79	15.94±0.42
RBC count ($x10^6$/µl)	Males (n=5)	4.92±0.23	4.68±0.25	4.72±0.19	4.84±0.34
	Females(n=5)	5.52±0.22	6.26±0.17	6.44±0.26	6.06±0.41
WBC count ($x10^3$/µl)	Males (n=5)	8.84±0.70	9.56±0.61	9.90±0.41	8.46±0.60
	Females(n=5)	10.48±0.48	9.12±0.60	9.36±0.57	9.10±0.38
Platelet count ($x10^3$/µl)	Males (n=5)	806.32±38.21	813.99±41.88	797.31±53.46	806.25±36.90
	Females(n=5)	834.31±39.97	859.00±42.11	817.15±38.96	830.45±40.24
Hematocrit (%)	Males(n=5)	47.42±2.40	47.94±2.36	46.36±1.83	45.46±2.11
	Females(n=5)	42.06±1.05	42.70±1.22	41.84±0.81	42.22±0.75

Neutrophils (%)	Males (n=5)	46.72±2.05	50.06±2.45	53.06±2.13	52.80±2.22
	Females(n=5)	45.24±1.68	45.64±1.26	47.36±2.01	47.80±1.66
Lymphocytes (%)	Males(n=5)	33.64±1.96	28.12±1.67	30.74±2.30	33.01±2.95
	Females(n=5)	34.26±1.65	30.29±2.45	30.32±2.68	34.40±2.17
Eosinophils (%)	Males(n=5)	2.76±0.44	3.68±0.25	3.52±0.46	3.02±0.38
	Females(n=5)	3.12±0.44	2.74±0.33	2.16±0.31	3.18±0.43
Monocytes (%)	Males(n=5)	5.42±0.74	5.90±0.49	5.86±0.60	4.48±0.34
	Females(n=5)	3.52±0.24	2.84±0.52	3.04±0.45	4.14±0.35
Basophils (%)	Males(n=5)	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
	Females(n=5)	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00

Values are expressed as mean±SEM, n=5 females and 5 males.

Table 6 : Effect Of Ethanolic Extract of *Celtis Timorensis* (EECT) on Biochemical Parameters in Rats-Sub-Acute Toxicity Study

Biochemical Parameter	Sex	Treatment group			
		Control	250 mg/kg	500 mg/kg	1000 mg/kg
Creatinine (mg/dl)	Males (n=5)	1.38±0.32	1.52±0.17	1.48±0.16	1.61±0.15
	Females(n=5)	1.56±0.26	1.56±0.21	1.64±0.15	1.70±0.20
Glucose (mg/dl)	Males (n=5)	121.24±7.90	124.41±6.05	122.21±7.44	128.83±3.23
	Females(n=5)	121.80±6.73	125.23±4.94	114.98±7.92	121.41±6.83
Total protein (g/dl)	Males (n=5)	7.34±0.29	6.24±0.19	6.02±0.50	6.26±0.46
	Females(n=5)	7.10±0.31	6.04±0.15	5.52±0.28	6.22±0.29
ALP (U/l)	Males (n=5)	78.78±5.10	78.96±3.03	91.62±2.65	90.96±3.50
	Females(n=5)	79.5±6.04	88.26±7.10	89.20±4.51	100.60±6.80
AST (U/L)	Males(n=5)	55.20±3.12	54.80±3.33	51.06±4.29	54.57±4.32
	Females(n=5)	52.36±2.38	54.02±3.64	48.48±3.98	51.60±4.10
ALT (U/L)	Males (n=5)	41.21±3.29	45.57±2.61	47.38±2.65	43.81±2.63
	Females(n=5)	40.02±3.76	44.22±2.00	47.48±2.29	40.58±3.19
BUN (mg/dl)	Males(n=5)	29.02±2.03	29.78±2.59	25.81±2.26	26.21±3.23
	Females(n=5)	27.68±2.80	29.24±1.41	24.96±3.23	27.04±3.07
Triglycerides (mg/dl)	Males(n=5)	64.81±2.63	59.79±4.93	70.18±3.59	58.23±5.58
	Females(n=5)	67.58±2.46	64.17±4.23	65.79±1.93	59.36±5.03
Total cholesterol (mg/dl)	Males(n=5)	83.01±4.76	75.62±7.67	74.38±4.91	70.22±6.85
	Females(n=5)	79.58±4.41	73.24±7.33	71.61±5.35	64.42±5.64
Albumin (g/dl)	Males(n=5)	3.72±0.18	4.20±0.24	4.42±0.39	3.90±0.29
	Females(n=5)	3.84±0.22	4.26±0.25	4.44±0.30	4.01±0.33
T.Bilirubin (mg/dl)	Males(n=5)	0.52±0.18	0.76±0.15	0.70±0.12	0.52±0.19
	Females(n=5)	0.80±0.15	0.74±0.20	0.58±0.19	0.62±0.16

Values are expressed as mean±SEM, n=5 females and 5 males.

IV. Discussion

In developing countries, herbal products prepared from medicinal plants have become famous in healthcare and some have been falsely considered as safe as they are obtained from natural sources. Nevertheless, these bioactive compounds from traditional medicinal plants are concluded to be safe without understanding the possible health effects and thus commonly used as self medication [11]. However, there is a lack of data on the toxicological profile and adverse effects of these compounds. Therefore, acute toxicity study is required not only to identify the further range of doses in animal studies but also to explain the probable clinical signs evoked by the test compounds under investigation. It is also an important effective parameter for calculating the therapeutic index of drugs and chemicals [12]. Results obtained from toxicity studies on animals will be critical for positive judgement on the safety of medicinal plants if they are found to have adequate potential for development into pharmacological compounds [13]. As the use of plant based products increases, it is important to screen the toxicological profile of these plants to confirm the safety and efficacy of those natural sources. Hence the present study was undertaken to evaluate the acute and sub-acute toxicity of ethanolic extract of *celtis timorensis* (EECT).

Throughout the 14 days of observation period, no morbidity or mortality was observed in the extract treated mice. In the present study, the results showed no adverse events in the dose groups 2000 mg/kg and 5000 mg/kg which indicate that the LD50 was greater than 5000 mg/kg. The sub-acute dose was selected based on the rats LD50 value which kept rats alive, i.e. 1/5, 1/10 and 1/20 of 5000 mg/kg. In the repeated dose 28-day oral toxicity study, there were no deaths and treatment-related signs were observed in all the groups

of animals. After exposure to a few possible toxic substances, there will be changes in body weight gain and internal organ weights which would reflect toxicity [14]. The body weight changes are markers of adverse effects of drugs and chemicals and if the body weight loss occurred is more than 10% of the initial body weight it will be considered as statistically significant [14, 15]. Organ weight also is an important indicator of physiological and pathological status of animals. The relative organ weight is fundamental to confirm whether the organ weight was exposed to the injury or not. The heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicant [16]. There were no significant differences in body weight gain of both control and treated groups. In the present study, organ weights in all the treated groups of both sexes were not significantly different from those of control groups. Hence it can be concluded that EECT is almost non-toxic. It is also important to measure the food intake and water consumption during the study of the safety of a product with medicinal purpose, as proper intake of supplements is necessary to the physiological status of the animal and to the achievement of a better response to the test substance under investigation [17, 18]. In this study, the food intake and water consumption also was not affected by the administration of EECT and it did not promote any appetite suppression and had no unfavourable effects. Thus, this indicates there was no interruption in the metabolism of carbohydrate, protein and fat.

Analysis of blood parameters is important in the evaluation of risks associated with test compounds under investigation as the changes in the hematological system have a greater indicative value for human toxicity, when the data are converted from animal studies [19]. In the present study, treatment with EECT for 28 days did not produce any changes in hematological parameters (i.e. hemoglobin, platelet count, white blood cell count, red blood cell count) which indicate that the extract did not affect the blood cellular components or their production. Transaminases such as SGOT and SGPT are well known good indicators of liver function and used as biomarkers to conclude the probable toxicity of drugs and xenobiotics [20]. Normally, destruction to the liver parenchymal cells will result in an increase of both these enzymes in the blood [21]. There were no changes in the ALT and AST levels, which reveal that the extract did not affect the liver function/ or metabolism. Elevated bilirubin levels are an indication of altered liver functions and a small elevation is an important indicator of liver damage in laboratory animals or could be a sign of biliary duct obstruction. In order to assess the synthetic capacity of the liver, determination of plasma proteins like albumin is required and decrease in plasma proteins therefore tend to reflect chronic damage [22]. There were no significant differences in the levels of AST, ALT, bilirubin and total protein between the control and treated groups. These indicate that EECT did not cause any damage to the liver. The normal values of kidney parameters such as blood urea nitrogen (BUN) and creatinine suggest that sub-acute administration of EECT did not cause any damage to the kidney. Histopathological studies provide supportive evidence for biochemical and haemat-ological observations. No abnormality was recorded to histopathological examinations of all organs examined. Since there were no signs of toxicity with respect to hematology, biochemistry, organ weight, body weight and histopathological examination noted in all the tested groups, it can be inferred that EECT will not produce any toxicity. Based on the results, the No Observed Adverse Effect Level (NOAEL) of the extract is greater than 1000 mg/kg/day.

V. Conclusion

Treatment with single oral doses of 2000 mg/kg and 5000 mg/kg did not result in any toxic signs or mortality in the acute toxicity studies. Daily oral administration of ethanolic extract of *celtis timorensis* for a period of 28 days did not cause mortality, changes in body weight and body weight gain. Also, no significant changes in hematological, biochemical and histopath-ological alterations were observed at the end of the duration of the experiment. Hence, the no-observed adverse-effect level of the extract was found to be exceed 1000 mg/kg/day p.o. Overall, it can be concluded that the ethanolic extract was well tolerated in daily dose at 1000 mg/kg for a period of 28 days.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Efficacy of Bee Venom as an Anti-Viral Therapy for HCV Genotype 4

By Abd Elrazek M Ali Abd Elrazek & Usama M. Abdel-aal
Al-Azhar University, Egypt

Abstract- Use of traditional medicine is encouraged in many economically diverse countries when conventional medications fail. HCV prevalence is highest in Egypt at >10% of the general population, and China has the most people with HCV (29.8 million); approximately 52% of patients infected with HCV genotype 4 will develop chronic HCV. The use of interferon, currently the only approved therapy, is frustrating in many situations. Use of camel milk or drinking copious amounts of urine, moxibustion by fire, acupuncture and cupping, especially in the Saharan and Arabian areas, is currently popular in Egypt. Some traditional Egyptian medicine is related to Arabian, ancient Egyptian, or other religious beliefs. Most patients using traditional therapies show improvement over time in both clinical symptoms and laboratory results. Some showed SVR using Bee venom therapy.

Keywords: *bee venom, HCV, traditional, therapy.*

GJMR-B Classification : *NLMC Code: QV 268.5, QV 704*

Strictly as per the compliance and regulations of:

Efficacy of Bee Venom as an Anti-Viral Therapy for HCV Genotype 4

Abd Elrazek M Ali Abd Elrazek [α] & Usama M. Abdel-aal [σ]

Abstract- Use of traditional medicine is encouraged in many economically diverse countries when conventional medications fail. HCV prevalence is highest in Egypt at >10% of the general population, and China has the most people with HCV (29.8 million); approximately 52% of patients infected with HCV genotype 4 will develop chronic HCV.

The use of interferon, currently the only approved therapy, is frustrating in many situations. Use of camel milk or drinking copious amounts of urine, moxibustion by fire, acupuncture and cupping, especially in the Saharan and Arabian areas, is currently popular in Egypt. Some traditional Egyptian medicine is related to Arabian, ancient Egyptian, or other religious beliefs. Most patients using traditional therapies show improvement over time in both clinical symptoms and laboratory results. Some showed SVR using Bee venom therapy.

Keywords: bee venom, HCV, traditional, therapy.

I. Introduction

Globally, it was estimated that in 2005, more than 185 million people had hepatitis C virus (HCV) antibodies; (prevalence of 2.8 percent) HCV prevalence is highest in Egypt at >10% of the general population and China has the most people with HCV (29.8 million), [1,2] Most cases of acute hepatitis C are anicteric and asymptomatic, with fewer than 25 percent being clinically apparent. Fulminant hepatitis C is rare. Nevertheless, the long-term liability of acute hepatitis C is significant due to the high rate of chronic infection (HCV-RNA positive in 55 to 85 percent of cases) and chronic hepatitis (elevated serum ALT concentration in 60 to 80 percent of patients with chronic infection). Approximately 20 to 30 percent of those chronically infected will develop cirrhosis, and a proportion of those patients will develop hepatocellular carcinoma [3, 4, 5, 6]. Treatment with Peginterferon and weight-based ribavirin alone results in sustained virologic response rates of only 40 to 50 percent in patients with chronic HCV genotype 4 [7,8,9,]. Traditional medicines have been used for medicinal purposes for thousands of years. All major cultures including Native American, European, South American, Asian, and African cultures have used botanicals for healing purposes. As an example, saw palmetto was used for urinary symptoms in men from Egypt in the 15th century BC10 [10].

In this study we will shed light on one of these traditional medical therapies; Bee venom as an antiviral therapy for HCV genotype 4 in Egypt.

II. Patients and Methods

We evaluated traditional bee sting venom medicine and reviewed the cases of five Egyptian patients infected with HCV genotype 4. This group included middle-aged adults, four males and one female, with an average age of 43.6 years.

The average BMI was 28, and all patients had chronic HCV infection. Three patients had portal hypertension causing splenomegaly and porto-systemic collateral shunt vessels. Two patients had thrombocytopenia, thyroiditis, and arthralgia related to HCV infection. All patients were instructed to discontinue all medications except those for hypertension.

All patients were administered bee venom using the traditional Egyptian medicine protocol. A total of three to five bee stings were applied above the knee joint. This was performed three times per week for 3 months. Bee death following the procedure was evidence of successful venom injection.

III. Results

Surprisingly, one male patient showed a sustained viral response (SVR) by PCR following 3 months of treatment. Qualitative PCR was repeated 6 and 12 months following conclusion of the bee sting treatment protocol.

a) Clinical

1. The patient appeared healthier with an acceptable general condition.
2. No heat or pain in any joint.
3. No development of signs associated with hepatic decompensation.
4. No manifestations of other diseases (cardiac, dermatological, renal, or pulmonary).
5. disappearnce of all extrahepatic manifestations of HCV ; arthralgia, glucose Intolerance, Itching, and thyroids.

b) U/S Applications

1. Improvement of general cirrhotic morphology with less coarseness and elevated echogenicity of fat.

Author α: Department of Liver Transplant and Hepatology-Al-Azhar Faculty of Medicine-Egypt. e-mail: ahmadrazek@gmail.com
Author σ: Department of Internal Medicine-Sohag Faculty of Medicine-Egypt.

2. Decreased spleen diameter.
3. Decreased portal vein diameter.
4. Decreased splenic vein diameter.

c) Laboratory Values

1. We estimated PCR only in 3 patients.One male patient showed SVR, another patient showed a significant (2 log) decrease in viral load;(both patients had early cirrhosis without portal hypertension or extrahepatic manifestation) Whatever the third patient (Alcoholic with advanced degree of liver cirrhosis and portal hypertension) showed a decrease of only 1 log. (Diagram 1)
2. The blood glucose profile normalized in patients with impaired glucose tolerance.
3. The thrombocytopenia improved, but did not return to the normal range.

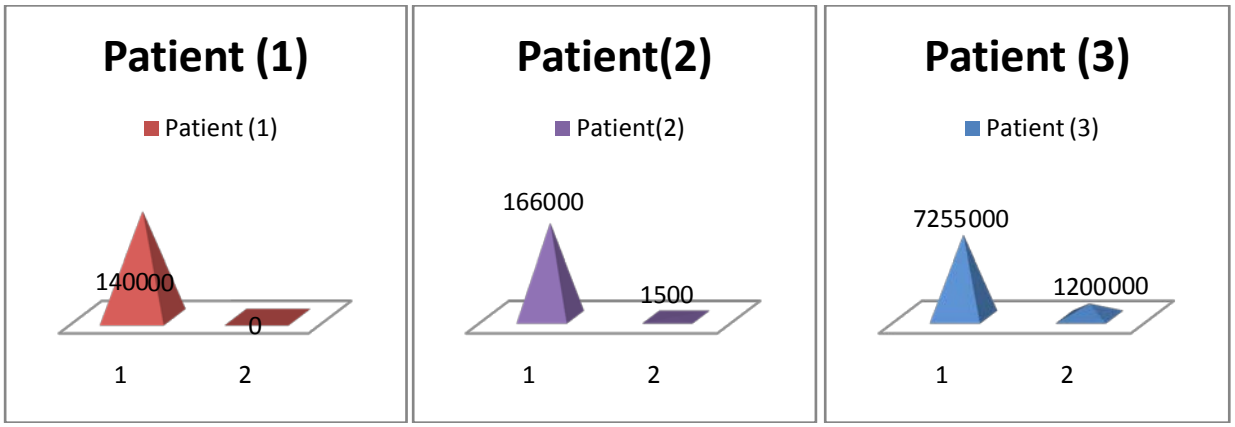

Diagram (1) : Showing PCR significant and insignificant decreased Log in patients 2&3 respectively. Patient (1) showed SVR

Table 1 : showing Liver enzymes pre and post Venom Therapy. There is also evidence of ALT, AST improvement post Venom therapy. (Normal ALT, AST values); *Up to 40 and 45 respectively.* (Normal PCR); *Undetectable viremia by TMA Test.*

		BEFORE BEE VENOM THERAPY			AFTER BEE VENOM THERAPY		
Sex	Age	ALT	AST	PCR	ALT	AST	PCR
M	24	48	47	*140,000*	30	31	*SVR*
M	34	50	52	166,000	24	35	1500
M	44	65	55	*7,255,000*	25	33	*1,200,000*

IV. Discussion

Use of traditional medicine is encouraged in many economically diverse countries when conventional medications fail.

Disease progression in patients undergoing conventional treatment causes frustration and feelings of oppression and defeatism, which can lead to depression. This is particularly true for highly intellectual individuals. Depression has significant effects on the immune system, and increases morbidity/mortality, resulting in serious adverse events and multisystem infection. Disease progression is directly related to the effect of depression on the immune system. The patient is then conflicted, as he must fight a hidden enemy, the virus, within his body. This creates a potentially tragic scenario.

Egypt has the highest prevalence of chronic hepatitis C virus (HCV) in the world; >10% of the population is infected. Most cases of acute hepatitis C are asymptomatic, with fewer than 25 percent being clinically apparent. Fulminant hepatitis C is rare. Nevertheless, the long-term liability of acute hepatitis C is significant due to the high rate of chronic infection (HCV-RNA positive in 55 to 85 percent of cases) and chronic hepatitis (elevated serum ALT concentration in 60 to 80 percent of patients with chronic infection). Approximately 20 to 30 percent of those chronically infected will develop cirrhosis, and a proportion of those patients will develop hepatocellular carcinoma. The use of interferon, currently the only approved therapy, is frustrating in many situations. This is because coinfection with Schistosoma spp., genotype resistance, complications of interferon/ribavirin therapy, long

treatment courses, and relapses, lead to poor outcomes compared to infection with genotypes 2 and 3 [11,12.13]. In patients with significant fibrosis, our approach depends upon the patient's HCV genotype. In patients HCV genotype 1 or 4, guidelines recommended IL28B genotype to determine if the patient is likely to respond to therapy. Accordingly we prefer to withhold antiviral therapy if the patient does not have a favorable genotype (CC at the rs12979860 polymorphic site). However, in an otherwise healthy patient with HCV genotype 1 or 4 and a favorable IL28B genotype, treatment is reasonable with close monitoring for side effects. Unfortunately many HCV genotype 4 patients have not the favorable genotype, especially those live in Egypt.

Use of traditional Egyptian therapy has been strongly encouraged in the last 10 years throughout Egypt.

Use of camel milk or drinking copious amounts of urine with or without moxibustion by fire, acupuncture and cupping, especially in the Saharan and Arabian areas, is currently popular in Egypt (figure 1).

Figure 1 : showing different traditional medicine used in Egypt especially in Saharan and Sub-Saharan regions for the therapy of chronic diseases especially those with HCV genotype 4

Some traditional Egyptian medicine is related to Arabian, ancient Egyptian, or other religious beliefs. Most patients using traditional therapies show improvement over time in both clinical symptoms and laboratory results. Patients have been found to have significantly decreased ascetic fluid volume or resolution of ascites, improved libido and stamina, improved renal function tests (particularly excretion function), and a significantly decreased viral load.

In the current study we evaluated traditional bee sting venom medicine and reviewed the cases of five Egyptian patients infected with HCV genotype 4;(4 males and 1 female), with 43.6 average age and 28 average calculated BMI, surprisingly one male patient showed a sustained viral response (SVR) by PCR following 3 months of treatment, qualitative PCR was repeated 6 and 12 months following conclusion of the bee sting treatment protocol, all patients showed improvement in the general condition. Arthralgia, glucose intolerance, itching and tyroditis subsided accompanied with better sonographic pictures following 3 months of the therapy.

It is well known that Bee Venom consists of several biologically active peptides, including mellitin, apamin, adolapin and mast cell degranulating peptide [14]. Few studies have indicated that administration of Bee Venom can significantly impart an anti arthritic response and initiate the protective mechanism against hepatic fibrosis [15].

To our knowledge no reports mentioned the traditional therapeutic use of Bee Venom as an effective therapy against HCV genotype 4, but we need to know more about the mechanism of action, either through antiviral mechanism or immunostimulation therapy, whatever emerging of new drugs e.g.: Sofosbuvir and Simebrevir may be the clue for the therapy of HCV different genotypes.

V. Study Limitations

The response to traditional bee-venom-based medication should be further evaluated. The properties of the venom likely vary according to the flowers visited by, and the environment of, the bees.

We used our clinical knowledge to evaluate the results of this report.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Hyperuricemia in Type 2 Diabetes Mellitus

By Nagendra. S., Kashinath R. T. & Srinivas. S.
Rajiv Gandhi Health University, India

Abstract- Recently there has been a growing interest in the association of uric acid levels with hyperglycemia. Insulin deficiency or subnormal functioning of insulin may induce possible alterations in purine nucleotide metabolism, specifically uric acid turnover. Studies have indicated that a close relationship do exists between plasma uric acid levels and glucose utilisation in type 2 diabetes mellitus. Though there are reports showing elevated plasma uric acid levels in type 2 diabetes mellitus but the origin of raised uric acid is still obscure. Hence a study was undertaken to assess the origin of raised plasma uric acid levels in diabetes mellitus. The type 2 diabetic subjects attending the OPD of Subbaiah Medical College Hospital, Purale, Shimoga were randomly selected. A fasting Blood sample was collected and the plasma samples were employed for estimation of glucose, uric acid, adenosine deaminase and 5'-nucleotidase levels. The results indicate a parallel raise in the plasma levels of adenosine deaminase and in 5'-nucleotidase along with plasma uric acid levels in type 2 diabetic subjects suggesting the raised plasma uric acid in type 2 diabetic subjects is due to increased purine catabolism.

Keywords: *type 2 diabetes mellitus, plasma uric acid, ada, 5'-nucleotidase.*

GJMR-B Classification : *NLMC Code: WD 200, WK 810*

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Keywords: type 2 diabetes mellitus, plasma uric acid, ada, 5'-nucleotidase.

I. Introduction

Insulin deficiency as observed in type-2 diabetes mellitus apart from inducing disturbances in glucose and fat metabolism may also cause possible alterations in nucleotide metabolism, specifically in uric acid turnover. Uric acid, the end product of purine metabolism, is produced by the degradation of purine nucleotides and purine nucleosides with the help of degradativeenzymes, 5' Nucleotidaseadenosinedeaminase, nucleosidephosphorylase and xanthine oxidase. Since the time our pioneer observation regarding the raised blood uric acid levels in diabetic subjects (1), many reports have appeared showing a relationship of plasma uric acid levels with hyperglycemia (2-17). Many research workers (2-15) suggest a positive correlation between plasma uric acid levels and diabetes mellitus while few reports advocate no such correlation (16,17). The specific observation of Feldmann & Lebrovitz (18), that ammonium ion (NH_4^+) do modulate the glucose induced insulin secretion /action relates nucleotide metabolism to insulin action, as ammonia is a bye-product of purine nucleotide degradation.

Hence a study was planned to reassess the plasma uric acid levels in diabetic subjects as well as to establish the possible origin of the raised plasma uric acid levels in type 2 diabetic subjects.

II. Materials and Methods

All the chemicals and reagents employed in the present study were of analar grade, and the adenosine as well as AMP (Adenosine mono phosphate) (kindly donated by Dr. Aski, B M Patil Medical College, BLDE University, Bijapur, Karnataka, India) were of chromatographic purity.

The type 2 diabetic subjects(both sexes) attending the medical OPD of Subbaiah Medical College Hospital ,Purle, Shimoga, who were in the age group of 30-60 years were randomly selected. Age matched normal subjects were selected from the employees of medical college and from medical college hospital. The subjects having orthopedic problems were excluded from the study. A fasting blood sample from both the normal as well as diabetic subjects were collected (4-5ml) with heparin as an anticoagulant after obtaining an informed consent from them. These blood samples were centrifuged for about 6-8 minutes at 3500rpm.

The separated clear plasma was employed for estimation of glucose (19), uric acid (20), Adenosine deaminase (ADA) (21) and 5'-Nucleotidase (22) levels. The results obtained were statistically analysed and the significance were calculated using Student't' test.

III. Results

A total number of 224 subjects including 120 diabetic and 104 normal subjects were employed in the present study. The diabetic subjects included 72 male diabetics and 48 female diabetic subjects. The normal subjects included 60 male and 44 female subjects. These diabetic subjects when divided age wise, there were 52 diabetic subjects in the age group of 30-50years and 68 diabetic subjects were above the age of 50years. Further these diabetic subjects were including 61 diabetics with positive family history of diabetes and 63 without family history of diabetes. This distribution of subjects are given in chart 1. The results obtained in the present study are depicted in table 1 to table 6.

Table 1 narrates the plasma levels of glucose, uric acid, ADA and 5'-Nucleotidase in normal subjects and in type 2 diabetic subjects. It is evident from the table that a significant raise is seen in plasma levels of

Author α: Department of Medicine, Subbaiah Institute of Medical Sciences & Research Center NH-13, Purale. Shimoga.
e-mail: smcshimoga@yahoo.co.in
Author σ: Department of Biochemistry, Subbaiah Institute of Medical Sciences & Research Center, NH-13, Purale. Shimoga.
e-mail: drkashinath_1945@yahoo.co.in
Author ρ: Department of Dermatology, Subbaiah Institute of Medical Sciences & Research Center NH-13, Purale. Shimoga.
e-mail: skin.srinivas@yahoo.com

uric acid(P>0.001), ADA (P>0.001), and 5'-Nucleotidase (P>0.001), in diabetic subjects as compared to normal subjects suggesting that the raise in uric acid level is due to increased degradation of purine nucleotides & nucleosides.

Table 1 : Table showing the levels of glucose, uric acid, 5'-nucleotidase and adenosine deaminase in plasma in normal and diabetic subjects

	Glucose mg. %	Uric acid mg.%	Adenosine deaminase units/L.	5'-Nucleotidase units/100ml.
Normal Subjects (104)	78.6 ± 16.4	5.80 ± 1.20	12.62 ± 4.22	7.0 ± 0.08
Diabetic Subjects (120)	226.40*** ± 28.3	11.8*** ± 2.10	28.12*** ± 8.61	37.5*** ± 11.25

Note: 1. The number in parenthesis shows the number of samples
2. Values are expressed as their Mean + S
*3. p- value*p<0.05, *p<0.01, *** p< 0.001.*

Table 2 gives the plasma levels of glucose, uric acid,ADA and 5'-Nucleotidasein normal male subjects and in type 2diabetic subjects. It is clear from the table that all the parameters studied are significantly elevated in male diabetic subjects as compared to normal male subjects(p>0.001).

Table 2 : Table showing the plasma levels glucose, uric acid, adenosine deaminase, 5'-nucleotidase in normal male subjects and type 2 diabetic male subjects.

	Glucose mg/dl	Uric acid mg/dl	Adenosine deaminase units/L.	5'-Nucleotidaseunits/100ml.
Normal male subjects (60)	72.20 ± 12.42	5.62 ± 1.18	12.20 ± 3.60	6.8 ± 1.0
Diabetic male subjects (72)	208.80*** ± 16.12	10.82*** ± 2.22	27.90*** ± 7.80	36.0*** ± 9.0

Note: 1. The number in parenthesis shows the number of samples
2. Values are expressed as their Mean + SD
*3. p value*p<0.05, *p<0.01, *** p< 0.001.*

Table 3 gives the plasma levels of glucose, uric acid,ADA and5'-Nucleotidase in normal female subjects and in type 2diabetic female subjects. It is evident from the table that all the parameters studied are significantly elevated in diabetic female subjects as compared to normal female subjects(p>0.001).

Table 3 : Table showing the plasma levels glucose, uric acid, adenosine deaminase and 5'-nucleotidase in normal female subjects and type 2 diabetic female subjects

	Glucose mg/dl	Uric acid mg/dl	Adenosine deaminase units/L.	5'-Nucleotidase units/100ml.
Normal female subjects (44)	74.80 ± 6.80	5.62 ± 1.22	11.80 ± 2.10	7.0 ± 2.2
Diabetic female subjects (48)	212.62*** ± 12.20	11.30*** ± 1.80	28.20*** ± 6.60	37.1*** ± 6.60

Note: 1. The number in parenthesis shows the number of samples
2. Values are expressed as their Mean + SD
*3. p- value*p<0.05, *p<0.01, *** p< 0.001.*

Table 4 gives the plasma levels of above parameters in type 2 male diabetic subjects & in type 2 female diabetic subjects. It is clear from the table that no variation in the parameters studied were observed in male diabetics ascompared to femalediabetic subjects.

Table 4 : Table showing the variation of glucose , uric acid, adenosine deaminase and 5'-nucleotidase in plasma in diabetic male subjects and diabetic female subjects

	Glucose mg. %	Uric acid mg.%	Adenosine deaminase units/L.	5'-Nucleotidase units/100ml.
Diabetic male Subjects (72)	208.80 ± 16.12	10.82 ± 2.22	25.84 ± 5.36	36.0 ± 9.00
Diabetic Female subjects (48)	212.62 ± 12.20	11.30 ± 1.80	28.20 ± 6.60	37.10 ± 6.60

Note: 1. The number in parenthesis shows the number of samples
2. Values are expressed as their Mean + SD
*3. p- value*p<0.05, *p<0.01, *** p< 0.001.*

Table 5 & Table 6 narrates the plasma levels of glucose, uric acid,ADA and 5'-Nucleotidase in diabetic subjects of 30-50years of age group and in diabeticsubjects above the age of 50years(Table 5)as well as in diabetic subjects with positive family history of diabetes mellitus and in diabeticsubjects without any family history of diabetes mellitus (Table 6). As seen from the tables no significant variations observed between diabeticsubjects of different age groups as well as between the diabeticsubjects with positive family history of diabetes mellitus as compared to diabeticsubjects without any such diabetic history.

Table 5 : Table showing the variation of glucose , uric acid, adenosine deaminase and 5'-nucleotidase in plasma in diabetic subjects with different age group

Age Group	Glucose mg. %	Uric acid mg.%	Adenosine deaminase units/L.	5'-Nucleotidaseunits/100ml.
30-50 Years (52)	210.6 ± 16.8	11.7 ± 3.10	25.02 ± 4.82	27.0 ± 5.50
Above 50 Years (68)	222.4 ± 22.6	11.6 ± 3.32	22.88 ± 5.66	26.5 ± 6.00

Note: 1. The number in parenthesis shows the number of samples
2. Values are expressed as their Mean + SD
*3. p- value*p<0.05, *p<0.01, *** p< 0.001.*

Table 6 : Table showing the variation of glucose , uric acid, adenosine deaminase and 5'-nucleotidase in plasma in diabetic subjects with or without family history of Diabetes mellitus.

Age Group	Glucose mg. %	Uric acid mg.%	Adenosine deaminase units/L.	5'-Nucleotidase units/100ml.
Diabetics with family history (61)	208.8 ± 18.6	10.9 ± 2.80	28.12 ± 5.16	28.5 ± 6.90
Diabetics Without family history (63)	220.6 ± 22.8	10.8 ± 1.20	26.32 ± 4.12	30.5 ± 5.80

Note: 1. The number in parenthesis shows the number of samples
2. Values are expressed as their Mean + SD
*3. p- value*p<0.05, *p<0.01, *** p< 0.001.*

IV. Discussion

Starting with the first observation (1), showing the increased whole blood uric acid levels in diabeticsubjects, several reports have been presented suggesting a relationship between the uric acid levels and hyperglycemia in diabetic subjects (2-17). Many reports advocating a raise in plasma uric acid levels in diabetic subjects (2-15) while few negate such observation (16, 17). The significant enzymes, which are quite abundant in tissues, responsible for the purine degradation are Adenosinedeaminase (Adenosine amino hydrolase EC: 3, 5, 4, 4) and 5'- Nucleotidase (5' nucleotide phosphohydrolase EC: 3, 1, 3, 5). Adenosinedeaminase is implicated in inflammatory conditions as well as in micro and macro vascular complications of diabetes mellitus (23). Similarly 5' nucleotidase has been claimed elevated in type 2 diabetes mellitus (24). Adenosine mimics the action of insulin on glucose and lipid metabolism in adipose tissue as well as in myocardium, while it inhibits the insulin effect on total hepatic glucose output suggesting that adenosine causes local insulin resistance in liver tissue. Adenosine modulates the action of insulin on various tissues differently and its tissue concentration is affected by ADA levels (25, 26). A parallel rise in the enzyme activities of adenosine deaminase and 5'-Nucleotidase in plasma, which may be due to an increase in their levels in the tissues, along with a rise in plasma uric acid levels suggest that the rise in plasma uric acid observed in the present study in type 2 diabetic subjects may be due to increased degradation of purine nucleosides and nucleotides. Kurtul N etal (27)have shown increased level of serum ADA activity in type 2 diabetic subjects with its correlation to HbA1c and suggested that ADA is important enzyme for modulating the bioactivity of insulin.

Subnormal insulin levels or insulin resistance seen in type 2 diabetes mellitus may decrease the activity of many glycolytic and citric acid cycle enzymes as insulin is a known promoter of the activities of pyruvatedehydrogenase, hexokinase, phosphofructok-inase, pyruvatekinase, α-ketoglutaratedehydrogenase etc (28). Such a decrease in the activity of these enzymes leads to accumulation of glucose-6-phosphate, which may be channeled through HMP pathway causing an increase in ribose-5-phosphate which is the starting compound for purine biosynthesis. Thus purine synthesis increases resulting in an elevated formation of uric acid.

It is known that the end regulation of insulin action is achieved through regulating protein-tyrosine phosphstases (PTP) which are thiol enzymes (29, 30, 31). One of the optimistic speculation is that the tissues and cells do try to adjust to the insulin deficiency state by prolonging the insulin action through regulating these PTPs by generating little amount of free oxygen species and these oxygen species in turn try to slow down the activity of PTPs by reacting with their free thiol groups. A possible reaction to generate oxygen species is purine degradation. A rise in plasma uric acid levels seen in the present study in type 2 diabetic subjects do support this speculation. This rise in plasma uric acid levels in diabetic subjects may also due to deterioration of glucose metabolism which is primarily due to insulin insufficiency as it is suggested by many research workers that increased plasma uric acid levels do correlate with deterioration of glucose metabolism in type 2 diabetic subjects(32,33).

The rise in plasma uric acid levels in type 2 female diabetic subjects is more pronounced as compared to type 2 male diabetic subjects (ref table 4) is in agreement with the earlier reports (34, 35) and which may be due to estrogen, as estrogen is known to influence secretion of adrenal steroids which inturn influences the catabolism of nucleotides and nucleic acids (36, 37). No much variations are seen in the levels of uric acid, ADA and 5'-Nucleotidasein diabetic subjects of 30-50 yrs of age group as compared to diabetic subjects of above 50yrs age group (ref table 5) as well between diabetic subjects with positive family history as compared to diabetic subjects without any diabetic family history (ref table 6).

It is concluded from the results of the present study in type 2 diabetic subjects that there is a definite rise in plasma uric acid levels in these diabetic subjects as compared to their normal counterparts and the uric acid elevation is due to increased degradation of purines as evidenced by the rised activity of Adenosine deaminase and 5'-Nucleotidase.

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CHART -1

Chart showing the distribution of normal and diabetic subjects according to various parameters.

Total number of subjects in the present study	------224
Total number of normal subjects	----- 104
Normal males	------60
Normal females	----- 44
Total number of Type 2 diabetic subjects	------120
Male diabetes	------72
Female diabetes	------48
Diabetic subjects in the age group of 30—50yrs	------52
Diabetic subjects above 50yrs of age	------68
Diabetic subjects with positive family history of diabetes	------61
Diabetic subjects without positive family history of diabetes	----63

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Comparative Study of Different Brands of Alprazolam

By Dr. Safila Naveed & Fatima Qamar
Jinnah University for women Karachi, Pakistan

Abstract- Benzodizapenes are the most widely used anxiolytic drug. They have largely replaced barbiturates and meprobamate in the treatment of Anxiety because they are safer and more effective. Alprazolam belongs to this class of drugs, it is used for the treatment of the anxiety symptoms of panic disorders.Present study deals with a brief overview of the comparative study of different brands of Alprazolam tablets according to Pharmacopeia (BP) & United States Pharmacopeia (USP) . for this reason.3 differen brands of Alprazolam 0.5 mg tablets have been evaluated using quality control test of thickness, hardness, weight variation and friability to assess that whether these 3 brands are pharmaceutically equivalent.

Keywords: *thickness, hardness, weight variation and friability, alprazolam.*

GJMR-B Classification : *NLMC Code: QV 37, QV 38*

Strictly as per the compliance and regulations of:

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Abstract- Benzodizapenes are the most widely used anxiolytic drug. They have largely replaced barbiturates and meprobamate in the treatment of Anxiety because they are safer and more effective. Alprazolam belongs to this class of drugs, it is used for the treatment of the anxiety symptoms of panic disorders.Present study deals with a brief overview of the comparative study of different brands of Alprazolam tablets according to Pharmacopeia (BP) & United States Pharmacopeia (USP) . for this reason.3 differen brands of Alprazolam 0.5 mg tablets have been evaluated using quality control test of thickness, hardness, weight variation and friability to assess that whether these 3 brands are pharmaceutically equivalent.

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I. Introduction

Alprazolam is a triazolobenzodiazepine. It is used in anxiety states and panic disorders [1, 2]. Benzodiazepines are commonly used for deliberate self poisoning. It has been observed that in approximately one third of cases of deliberate self poisoning benzodizapenes are being used[3]. Alprazolam is a newer benzodiazepine and is used more commonly in overdose and no previous series of alprazolam poisonings were found. 14 case reports have been published including 5 reported deaths,2 in which alprazolam was ingested alone [4]. According to reports published by American Association of Poison Control Centers National Data Collection System indicated that alprazolam was involved in thirty four fatal deliberate self poisonings over ten years 1992–2001 compared with thirty fatal deliberate self poisonings involving diazepam [5]. This reveals significant alprazolam toxicity if prescribing practices in the United States (US) mirror Australian trends where diazepam is prescribed at five to ten times the rate of alprazolam. A British study showed the fatal toxicity index (deaths per million prescriptions) for diazepam was 4.0 compared with 5.9 for alprazolam. [6]. These data suggest that alprazolam is more toxic in overdose than other benzodiazepines.

In human liver this drug is metabolized by CYP3A enzymes to hydroxylated metabolites . 4-hydroxy alprazolam (4-OHALP) is the major and less active metabolite while α-Hydroxy alprazolam (α-OHALP) is the minor and active metabolite. There is an intrinsic difference in the biotransformation of alprazolam in liver and brain. As P450 enzymes present in brain is one-10th to one-15th of liver [7]

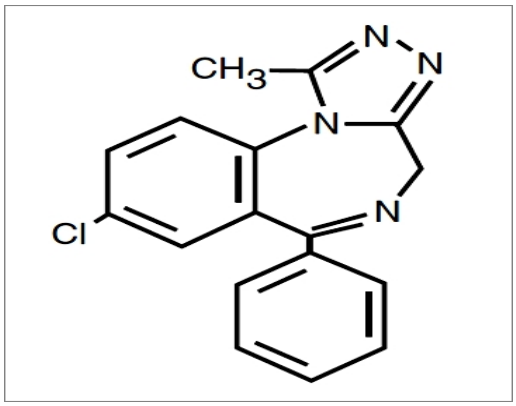

Figure 1 : Structure of alprazolam

II. Material and Methods

Alprazolam (0.5mg) tablets from the following thre different brands
Neuxam (stand pharmaceuticals)Batch P0315E
Alp (Hilton pharamceuticals) Batch 108891
Nervin (Werrick Pharmaceuticals) Batch 1971 Apparatus band equipments:

1. Analytical Balance
2. Rolex tablet hardness
3. Friability tester

The study used BP and other pharmacopeias to check the in vitro quality of alprazolam brands tablet using different analytical techniques and procedure described in the analytical techniques and procedures described in the pharmacopeias. For testing the physical parameters of alprazolam brands tablet Various instruments were used to measure content as well as qualities in general.

a) General Tests

Quality of alprazolam brands tablets were assessed in compliance with BP specifications. General tests include weight variation, tablets friability .

b) Weight Variation Test

Weight variation test of above mentioned tablets should be in accordance to the BP/USP requirements that not more than two tablets out of 20 tablets should cross ±7.5 % deviation.

Author α σ: Faculty of Pharmacy Jinnah University for women Karachi. e-mails: safila117@yahoo.com, fatimamudassar2009@hotmail.com

Table A : Limits for Uniformity of weight

Dosage form	Average weight	Percentage deviation
Uncoated and film coated tablets	80 mg or less	10
	More than 80 mg but less than 250 mg	7.5
	250 mg or more	5

c) Hardness

The official range of hardness stated in BP/USP is not less than 4.00 Kg of pressure is required to break a tablet, so all of the samples were tested for hardness.

d) Friability

To evaluate how well the tablets stands up to coating, packing, shipping and other processing friability test were conducted. From each brand of legal and illegal products, 20 tablets were taken and de dusted before weighing, after weighing the tablet were placed in drum of friability tester of which each tablet rotated 100 times. Finally 20 tablets of each brand were de dusted and reweighed and their percentage losses of weight were calculated. According to BP & USP the total weight loss should not be more than one percent and no tablet should show any type of break or crack.

Thickness Test: Thickness of above mentioned tablets including average , standard deviation, upper and lower limits are in accordance with BP/USP

III. Results

Weight Variation Test: Wt. variation test of alprazolam tablets proved statistically that all the tablets were in accordance to the BP/USP requirements. (Table-1 2 & 3)

Thickness Test: Thickness of all tablets of alprazolam including average,(SD) standard deviation and upper/ lower limits are in accordance with BP/USP (Table-4&5)

Hardness Test: Hardness test of alprazolam was found to not be in conjunction with the stated guidelines as given in BP/USP (Table-6&7).

Friabilty Test: Friability of alprazolam tablets was less than 1%. Therefore it is compliance with the BP/USP standards. It's data is given in (Table-8).

IV. Discusion

Uniformity of weight is compendia standard while hardness and friability are non compendia standards to assess the quality of the tablet. Friability is now included in USP.

a) Weight Variation

The table 1-3 indicate that weight variation values of Neuxam , Alp , Nervin showed that among three brands Alp has the highest value of the mean as compared to other brands. The requirements are met with weight variation qaccording to USP that is ,weight of not more than two tablets out of all brands differs from the average weight by more than 7.5%

b) Friability

The table 3 and figure 5(%weight loss for Nexaum , Alp , Nervin) showed that the Nexaum has the highest % weight lost and Alp has the lowest % weight lost when compared to Nervan.

All the three brands have less than 1% of weight lost which showed that these brands met the USP requirement.

c) Hardness

The official range of hardness stated in BP/USP is not less than 4.00 Kg of pressure is required to break a tablet, so all of the samples were tested for hardness.

The table and figure showed that Nervin has the highest hardness value and Alp has showed lowest hardness value. It shows that Alp showed to meet the USP requirement while the other two brands are beyond the USP limit.

Table 1 : Weight Of 20 Tablets (Randomly Selected) of Different Brands

Tablets	Neuxam	Alp	Nervin
1	179	195	165
2	173	199	158
3	180	200	150
4	178	200	156
5	180	202	159
6	190	200	156
7	190	203	151
8	178	205	156
9	179	198	160
10	176	205	154
11	173	200	160
12	176	195	158
13	180	199	151
14	177	203	156
15	173	198	150
16	179	202	165
17	180	200	156
18	190	199	154
19	190	202	158
20	176	200	160

Table 2 : Statistical Weight Variations

Tablets	Average (Gm)	Standard deviation	Upper limit (X+3S)	Lower limit (X-3S)
Neuxam	0.17985	0.005687	0.196911	0.162789
Alp	0.20025	0.002712	0.2083	0.19211
Nervin	0.15665	0.004308	0.16957	0.1437

Table 3 : Weight Variation Test

Tablets	Result (Gm)	BP/USP Specification	Deviation from BP/USP Specification
Neuxam Alp Nervin	0.17985 0.20025 0.15665	Deviation should be ±7.5%	Within specified limit

Table 4 : Thickness Of 10 Tablets (Mm)

Tablets	Neuxam	Alp	Nervin
1	3.3	3.2	3.2
2	3.4	3.1	3.2
3	3.3	3.2	3.2
4	3.2	3.1	3.2
5	3.5	3.1	3.1
6	3.3	3.1	3.1
7	3.3	3.1	3.1
8	3.5	3.1	3.1
9	3.4	3.1	3.1
10	3.3	3.1	3.1

Table 5 : Statistical Thickness

Tablets	Average Thickness mm	Standard deviation	Upper limit (X+3S)	Lower limit (X-3S)
Neuxam	3.35	0.09	3.64	3.05
Alp	3.12	0.04	3.246	2.99
Nervin	3.14	0.05	3.29	2.98

Table 6 : Hardness Of 10 Tablets From The Optimised Formulation

Tablets	Neuxam	Alp	Nervin
1	7.69	2.19	12.2
2	5.05	2.5	12.0
3	6.14	2.47	11.7
4	7.40	2.41	15.2
5	7.88	2.21	12.1
6	7.306	2.05	10.2
7	7.16	1.92	13.2
8	7.73	2.73	14.9
9	7.60	2.45	13.2
10	3.91	1.95	13.8

Table 7 : Statistical Hardness

Tablets	Average (Kg)	Standard deviation	Upper limit (X+3S)	Lower limit (X-3S)
Neuxam	6.7866	1.335725	10.79377	2.779426
Alp	2.288	0.265949	3.085847	1.490153
Nervin	12.85	1.524795	17.42439	8.275615

Table 8 : Friability Test

no. of tablets	Result (%)	BP/USP Specification	Deviation from BP/USP Specification
Neuxam	0.615	Not more than1%	In specified limit
Alp	0.099		
Nervin	0.128		

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Collection, Detection, Assessment, Monitoring and Prevention of Adverse Drug Reactions in the Nephrology Department of Gauhati Medical College and Hospital, Assam, India

By Prudhivi Ramakrishna, AK Barman, PJ Mahanta, Mangala Lahkar & Maddi Ramaiah

Abstract- An adverse drug reaction (ADR) as defined by World Health Organization (WHO) is a noxious, unintended effect of a drug, occurring at normal doses in humans for prophylaxis, diagnosis or therapy of disease or for the modification of physiological function. ADRs are considered as the fourth to sixth leading cause of death among hospitalized patients. About 2.9-5.6% of all admissions are caused by adverse related events, and approximately 35% hospitalized patients experience an ADR.

Objective: To identify the ADR by chart review method, to determine the causality of the ADR by Naranjo's algorithm, to analyze the severity of the ADR by modified Hartwig method and to motivate the health care professionals to report ADRs in Nephrology ward of Gauhatu Medical College and Hospital (GMCH), Guwahati. Preventability of ADR is done by Schumock &Thortonpreventibility scale.

Materials and methods: A prospective observational and hospital based case control study(June 2011-May 2012)was carried out in the Nephrology ward of GHMC, including both out-patient and in- patient departments. All the values are statistically determined using parametric t-test and non-parametric fisher's exact test or chi-square tests.

Keywords: *nephrology, renal dysfunction, moon face, hypersensitivity, hepatotoxicity.*

GJMR-B Classification : *NLMC Code: QV 37.5, QV 752*

COLLECTIONDETECTIONASSESSMENTMONITORINGANDPREVENTIONOFADVERSEDRUGREACTIONSINTHENEPHROLOGYDEPARTMENTOFGAUHATIMEDICALCOLLEGEANDHOSPITALASSAMINDIA

Strictly as per the compliance and regulations of:

Collection, Detection, Assessment, Monitoring and Prevention of Adverse Drug Reactions in the Nephrology Department of Gauhati Medical College and Hospital, Assam, India

Prudhivi Ramakrishna [α], AK Barman [σ], PJ Mahanta [ρ], Mangala Lahkar [ω] & Maddi Ramaiah [¥]

Abstract- An adverse drug reaction (ADR) as defined by World Health Organization (WHO) is a noxious, unintended effect of a drug, occurring at normal doses in humans for prophylaxis, diagnosis or therapy of disease or for the modification of physiological function. ADRs are considered as the fourth to sixth leading cause of death among hospitalized patients. About 2.9-5.6% of all admissions are caused by adverse related events, and approximately 35% hospitalized patients experience an ADR.

Objective: To identify the ADR by chart review method, to determine the causality of the ADR by Naranjo's algorithm, to analyze the severity of the ADR by modified Hartwig method and to motivate the health care professionals to report ADRs in Nephrology ward of Gauhatu Medical College and Hospital (GMCH), Guwahati. Preventability of ADR is done by Schumock & Thortonpreventibility scale.

Materials and methods: A prospective observational and hospital based case control study(June 2011-May 2012)was carried out in the Nephrology ward of GHMC, including both out-patient and in- patient departments. All the values are statistically determined using parametric t-test and non-parametric fisher's exact test or chi-square tests.

Results: Out of 850 patient records, thecommonly occurring ADRs were moon face (n=16, 18.6%) followed by hypersensitivity (n=9, 10.4%) and hepatotoxicity (n=4, 4.65%).Gastrointestinal ADRs were highest in number followed by the hypersensitivity. Prednisolone was found to be the most offending drug followed by Nimesulide and Diclofenac. It is very clear that 12.7% ADRs were preventable.

Conclusion: Renal dysfunction plays a significant role in occurrence of serious and multiple ADRs. Poly-pharmacy, co-morbidity and number of diagnosis were found to be risk factors for ADRs.

Keywords: nephrology, renal dysfunction, moon face, hypersensitivity, hepatotoxicity.

Author α, ¥: Department of Pharmacy Practice, Hindu college of Pharmacy, Guntur -522002, A.P., India.
e-mail: rampharma83@gmail.com
Author σ ρ: Department of Nephrology, Gauhati Medical College and Hospital, Guwahati-781032, Assam, India.
Author ω: Department of Pharmacology, Gauhati Medical College and Hospital, Guwahati-781032, Assam, India.

I. Introduction

An adverse drug reaction (ADR) as defined by World Health Organization (WHO) is a noxious, unintended effect of a drug, occurring at normal doses in humans for prophylaxis, diagnosis or therapy of disease or for the modification of physiological function(1). ADRs are considered as the fourth to sixth leading cause of death among hospitalized patients. About 2.9-5.6% of all admissions are caused by adverse related events, and approximately 35% hospitalized patients experience an ADR. ADRs are associated with significant morbidity, permanent disability and are a huge economic burden on patients due to prolonged hospitalization(2).

Kidney is the primary route of elimination for drugs and their metabolites. Hydrophilic drugs are mainly cleared by the kidney(3). ADRs are most commonly observed in elderly patients(4). Aging is associated with decreased renal and liver reserve and with the risk of delayed renal and hepatic clearance of drugs. Renal function can be readily estimated by the serum creatinine level(3).

The Gauhati Medical College and hospital (GMCH) has enjoyed a prestigious status in the country for its academic pursuites and patients care, and thereby being a referral centre for speciality and superspeciality treatment having a bed strength of 1,587 and 17 operation theaters It provide promotive, preventive and curative, through out-patient department (OPD), indoor, emergency and extension Services. An ADR reporting program exists in the hospital since 1970 and the same was coordinated by the department of pharmacy practice, National Institute of Pharmaceutical Education and Research (NIPER), Gauhati. The present study was undertaken to characterize the ADRs reported in Nephrology department(5).

II. Materials and Methods

The study was carried out in the Nephrology ward of GHMC from June 2010 to May 2011 including both out-patient (OP) and in-patient (IP) departments.

The study was a prospective observational, hospital based case control study.It was based only on those patients who experience an adverse reaction to medicine use, either during their stay in hospital or outside the hospital and visiting the outpatient and inpatient departments of Nephrology.

The degree of association of an adverse effect with a drug is done (table 1)with the help of Naranjo's algorithm where it involves a number of questionnaires, to each of which score has been provided (ranging from -1 to +2). Total score for a particular drug-ADR combination is calculated and the association is termed as>9: Highly probable; 5-8: Probable; 1-4: Possible; 0: Doubtful(6).

After the causality assessment has been done, the severity of the ADR is analyzed using adapted Hart wig severity scale(7).The scale was classified as mild:a reaction that does not required treatment or prolongation of hospital stay; moderate: a reaction that requires treatment and or prolongs hospitalization by at least one day; severe: a reaction that was potentially life threatening or contributes to the death of patient was permanently disabling requires intensive medical care or results in a congenital anomaly cancer or unintentional overdose.

Preventability of ADR is done by Schumock & Thorton preventibility scale. Preventable adverse drug reaction was defined according to Schumock and Thornton (1992) as ADR which was preventable or avoidable. There were seven questions. Answering "YES" to one or more of the questions that an ADR was preventable (8).

To study the onset of ADR, acute: those which are observed within 60 minutes after the administration of medication; sub-acute: those occur within 1-24 hours from the time of administration of medication; and latent: those take 2 or more days to become apparent, parameters were used.

III. Statistical Analysis

Data were recorded on a pre-designed proforma and managed on an MS Office Excel spread sheet. The descriptive statistics are represented by mean ± standard deviation and percentages. The differences between the groups were determined by the parametric t-test and non-parametric fisher's exact test or chi-square tests wherever appropriate. Graph Pad InStat version 3.12 statistical software was used for the data analysis. The Odds ratio and its 95% confidence interval were calculated for certain risk factor of ADRs in renal failure patients. Statistical significance was defined as $p<0.05$. All P values were two tailed.

IV. Results

The results were based on 850 patient records taken from the Nephrology department of GHMC. Out of them 72 (8.47%) patients resulted in one or more ADRs. Thecommonly occurring ADRs were moon face (n=16, 18.6%) followed by hypersensitivity (n=9, 10.4%) and hepatotoxicity (n=4, 4.65%)(Table2).

V. Types of Adrs by System

Gastrointestinal ADRs were highest in number followed by the hypersensitivity ADRs. Gastrointestinal ADRs mainly include hepatotoxicity, ulcers, melaena, nausea, vomiting diarrhea and constipation (table 2).

Table 2 : List of ADRs reported during study period

S.No	ADRs			
	Description	Frequency (%)	System wise	Frequency (%)
1	Moon face	16(18.6)	Gastrointestinal disturbances	18(20.9)
2	Allergic reactions	9(10.4)	Hypersensitivity	9(10.4)
3	Fluid electrolyte imbalance	4(4.65)	Ophthalmic	8(9.3)
4	Hepatotoxicity	4(4.65)	Cardiovascular	7(8.13)
5	Tachycardia	3(3.40)	Dermatological	5(5.81)
6	Melaena	3(3.40)	Respiratory	4(4.65)
7	Tremor	3(3.40)	Electrolytic	4(4.65)
8	Constipation	3(3.40)	Central nervous system	3(3.4)
9	Cataract	3(3.40)	Hematological	3(3.4)
10	Blurred vision	3(3.40)	Endocrinal	1(1.16)
11	Others	33(45.83)	Others	24(27.9)

The causality assessment was done using Naranjo's scale and it shows that majority of the ADRs were probable (n= 87, 91.57%). As the ADRs had been identified, their severity level was also assessed. This was done using Hartwig criteria and majority of the patients had mild ADR (n = 44, 51.16%). Mostly ADRs have latent onset. It was very clear that 12.7% ADRs were preventable.The other 87.2% were not preventable because the susceptibility of these ADRs is still not defined and is a matter of research.This assessment is based on Schumoch and Thornton preventability criteria.In this study maximum number of ADRs found in one patient are 3.They are fluid electrolyte imbalance, blurred vision, hyperglycemia.

The incidence and certainty of ADRs in male and female were also studied. It was found that female populations showed a higher incidence of ADRs than in male populations (table 3).The percentage of ADRs was found out by dividing number of patients with ADRs of a particular gender by total number of patients of the same gender.

For gender, the p-value is 0.042 (<0.05). It shows that there is significant difference between occurrences of ADRs in different gender. In this study females were found to be more prone to ADR when compared to the male patients, similar to more other studies in the literature.

This study shows the incidence of ADRs with respect to age in which elderly patients(age >60) had a higher incidence of ADRs (15.00%). The patients in between the age of 0-18 yrs were found to have 9.37% and the age between 19-60 yrs had 7.98%(table 3). For age, the p-value is 0.001(<0.05). It shows that there is significant difference between occurrences of ADRs in different age groups. In this study patients above 60 years were found to be more prone to ADR when compared to other age groups.

Table 3 : Total interpretation of results

S. No	Variable		Total (n)	Patients with ADR	Patients without ADR	Prevalence of ADR	OR* (95% CI**)
			850	72	778	8.47%	
1	Age (yrs)	0-18	96	9	89	9.37%	1 (reference)
		19-60	714	57	657	7.98%	0.85(0.41-1.8)
		≥ 60	40	6	34	15%	1.74(0.57-5.27)
2	Sex	Female	360	32	328	8.88%	1 (reference)
		Male	490	40	450	8.16%	0.91(0.56-1.48)
3	Number of medications	≤ 5	200	18	182	9%	1 (reference)
		6-10	280	19	261	6.77%	0.73(0.41-1.3)
		≥11	370	35	335	9.45%	1.06(0.4-1.5)
4	Number of diagnosis	1	198	9	189	4.54%	1 (reference)
		2	300	28	272	9.33%	2.16(0.99-4.61)
		≥3	352	35	317	9.94%	2.31(1.1-4.9)

OR – odds ratio, CI- confidence interval.

The prescription pattern in case of each patient with ADR was studied and accordingly the patients were divided into 3 groups namely, those receiving 1-5 numbers of drugs;those receiving 6-10 numbers of drugs; and those receiving more than 10 drugs. It was seen that patients receiving more number of drugs (>10)had higher chances of developing ADRs(9.45%) (table3). A total of 450 medicines were prescribed in patients with adverse reactions. prednisolone was found to be the most offending drug followed by nimesulide and diclofenac (figure 1).

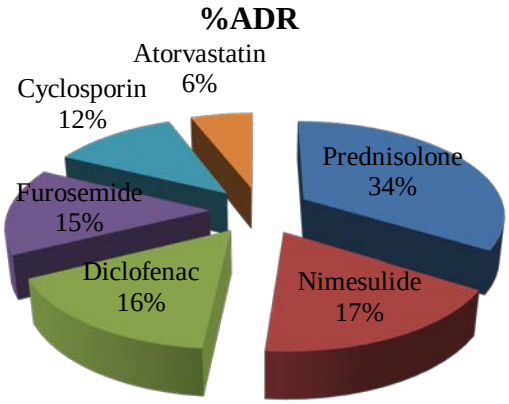

Figure 1 : Drugs causing ADRs

VI. Discussion

ADRs are the undesirable effects of the drug/ medicinal product beyond its intended therapeutic effect when used for clinical purposes. ADRs not only cause morbidity but also can be a reason for mortality in severe cases. They also contribute to greater extents in increasing health care costs to the patients and to the nation(9).

This was a prospective observational study carried out in the Nephrology department of GMCH. Out of the total 850 patient records collected in the Nephrology ward, 72patients resulted in one or more ADRs. Similar studies done in other countries reported a higher rate of incidence(10% to18%) than this study.

The commonly occurring ADRs in this study were moon face followed by Allergic reactions. This study finding was in accordance with the results of several other studies in literature(2, 4, 10).Where moon face was found to be the common ADR associated with prednisolone use.Causality assessment using Naranjo scale proved majority of the ADRs to be probably due to the drugs, while only 8.13%ADRs (table 1)were found to be possibly due to the drugs. Severity analysis using Hartwig scale showed majority of the patients had "mild" ADR (table 1).

Table 1 : Assessment of ADRs

Assessment	Criteria	No.of ADRs	% of ADRs
Naranjo's Score (causality)	Possible(1-4)	7	8.13
	Probable(5-8)	75	87.2
	Highly probable(>9)	4	4.65
Hartwig Criteria (severity)	Mild	44	51.16
	Moderate	38	44.18
	Severe	4	4.65

In this study females had a higher incidence of ADR as compared to the males. A higher incidence and more hospital admissions due to ADRs have been documented for women compared to men may be due to enhanced tissue sensitivity or the existence of sex-related differences in pharmacokinetics.

Patients above 60 years of age are more likely to develop ADRs and may even need hospitalization due to them. This study also showed a higher incidence of ADRs in the geriatric population when compared to the adults and pediatric age groups due to their modified pharmacokinetic and pharmacodynamics properties.

The number of patients visiting the IPD and OPD were recorded in this study. The incidence of ADR was found to be more frequently reported in the OPD setting than the IPD setting of the hospital.ADRs are very common in patients prescribed with poly therapy. In this study too it was observed that as the number of drugs prescribed increased, the cases of ADRs had also increased.

NSAIDs were implicated in a majority of ADRs (26.6%).Prednisolone was found to be the most offending drug followed by nimesulide and diclofenac. Previous Indian studies had documented non-opioid analgesics (18%) and Aminoglicosides(48%)(4).Mortality due to ADRs was 0.12% of the total admissions. The one death observed in the study was relatedto nimesulide induced melaena.The most common organ system associated with ADRs was GIT system followed by cutaneous reactions.

Some of the ADRs were preventable. For example, vancomycin injection caused finger necrosis due to a rapid injection. This ADR is avoidable by giving injection slowly.

VII. Conclusion

Some of the ADRs can be preventable by knowing clinical knowledge about drugs and their usage pattern. Elderly patients are at more risk of developing ADRs due to their modified pharmacokinetic and dynamic properties and renal dysfunction plays a major role in developing ADRs. Poly-pharmacy,age, co-morbidity and no. of diagnosis were found to be risk factors for ADRs.

So clinical pharmacists should be uptodate about their clinical knowledge and attend daily ward rounds with the Nephrologists in the hospital as part of the clinical services. All health care professionals should be encouraged to report the suspected Adverse Drug Events (ADE's) and have actively monitored those ADR's to ensure safe pharmacotherapy. A regular follow up of patients on drugs is required for the early detection and prevention of ADRs to increase patient's compliance to drug therapy and to provide a better drug therapy by prevention of related morbidity and mortality.

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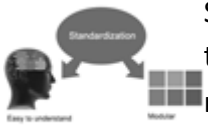

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Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

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

Materials:

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

Methods:

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

Approach:

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

What to keep away from

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

Results:

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

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

Content

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

What to stay away from

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

Approach

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

Figures and tables

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

Discussion:

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

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

Approach:

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

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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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ISSN 9755896