ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Sub-Acute Toxicological Evaluation of Roots of *Stereospermum suaveolens.*

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ABSTRACT

The present study was aimed to evaluate the sub- acute toxicity of ethanol extract of roots of *Stereospermum suaveolens* in Wistar rats. Ethanol extract of roots of *Stereospermum suaveolens* was prepared by hot extraction method. Acute toxicity studies had been carried out according to OECD 423 guidelines. Sub-acute toxicity studies were evaluated by orally administering the extract at dosage levels of 200 and 400 mg/kg b.w. for 28 days. At the end of the study blood was collected for haematological and biochemical specifications and animals were sacrificed and organ weights were monitored and subjected for histological studies. The results of the acute toxicity study shows that extract was safe at 2000mg/kg b.w. Sub-acute toxicity study did not show a significant change in any of the hematological and biochemical parameters. Further histological studies also substantiated the biochemical parameters. The findings of the present study conclude that the ethanol extract of roots of *Stereospermum suaveolens* was practically non toxic in rats after repeated oral administration. Further the study supports the use of roots of *Stereospermum suaveolens* in traditional systems of medicine.

Keywords: *Stereospermum suaveolens*, Acute toxicity, Sub-acute toxicity.

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ISSN: 0975-8585

INTRODUCTION

Utilization of medicinal plants in traditional system of medicine has been with man since antiquity. Till today, there is a widespread acceptance of herbal remedies in the developed and developing countries to complement conventional medicine [1] (Narahari et al., 2008). In the recent past, there has been a worldwide escalation in the usage of herbal remedies [2](Frass et al., 2012). This surge is attributed to the elevated cost of modern medicine, ready availability of traditional herbal remedies and inclination of the world population towards self-medication. Ordinarily, herbal remedies are considered as being safe, because they are regarded as natural and clinically safe based on their time tested usage for centuries[3,4] (Fennell et al., 2004; Haq, 2004). Furthermore, only a small number of medicinal plant species, their extracts or formulations have been pharmacologically and toxicologically validated.

Stereospermum suaveolens (F: Bignoniaceae), popularly known as padhri, is a medicinal plant widely used in traditional system of medicine. The various parts of the plant are used in Ayurveda and folklore medicine for the treatment of various ailments. Traditionally root is used in the remedies of diseases like in "kapha", and "amlapitta", inflammations, heating, dyspnoea, body ache, vomiting, eructation, piles, acidity, diarrhoea, gonorrhoea, loss of taste, liver disorders, malaria and other fevers. Root is also useful in excessive thirst, cough, asthma and weight gain [5](Krithikar and Basu 1988). Moreover the roots of *Stereospermum suaveolens* are reported to contain various chemical constituents like p-coumaric acid, triacontanol, cetyl alcohol, oleic, palmitic, stearic acid, lapachol, dehydroalpha- lapachone and dehydrotectol. Previous scientific investigations evidenced that plant possess anti-inflammatory, anticancer, hepatoprotective, antihyperglycemic, antioxidant activities [6](Meena et al., 2010).

Based on the ethnobotanical information and different activities reported with roots of *S. suaveolens*, the present study is aimed to ascertain its safety profile by acute and sub-acute toxicological evaluation in Wistar rats.

MATERIALS AND METHODS

Collection and authentication of plant material: The roots of *Stereospermum suaveolens* were collected from Tirumala hills of Chittor district of Andhra Pradesh. The plant was identified and authenticated by Botanist Dr. Madhavachetty, Herbarium keeper, Department of botany, Sri Venkateswara University, Tirupati, India and a specimen (No. 1587) has been deposited in Department of Botany, Sri Venkateswara University, Tirupati, India. Roots were shade dried and powdered in Wiley mill.

Preparation of ethanol extract: The root powder was defatted with petroleum ether (60-80^{0}C). The defatted marc was air-dried and macerated with ethanol for 24 h. Macerated material was refluxed for 3h., then filtered and subjected to distillation under reduced pressure to get the semi solid residue.

Animals: Wistar strain Albino rats weighing 150-180gm were selected for study. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. They had free access to standard pellets as basal diet and water *ad libtum*. The animals were acclimatized for 7 days before starting the experiment. The experimental protocol was approved by Institutional Animal Ethics Committee and carried out according to the guidelines of CPCSEA. (Registration No.: 1677/PO/a/12/CPCSEA).

Acute toxicity studies: The acute oral toxicity study was performed as per the Organisation for Economic Cooperation and Development (OECD) 423guidelines (OECD, 2001). Animals were administered with EESS at a dose of 2000mg/kg b.w. All the experimental animals were observed for their mortality and clinical signs of toxicity (general behavior, respiratory patterns, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 30 min, 2, 6 and 12 h and thereafter once a day for 14 days following the treatment [7].

Sub-acute toxicity studies:

Animals were randomly divided into three groups each group consisted of six rats. The first group was served as control and orally treated with vehicle for 28 days where as remaining two groups were orally treated with single dose of 200 and 400mg/kg b. w. respectively up to 28 days. Body weight gain and food intake were recorded during the experimental period. On day 29 of study, blood samples were collected by

retro-orbital puncture in two tubes: one with anticoagulant EDTA and other without anticoagulant. Rats were sacrificed by cervical decapitation and organs were dissected out and their weights were monitored.

Haematological parameters:

The anticoagulated blood was analyzed immediately for hematological parameters: erythrocytes (RBC), hemoglobin (HGB), platelet (PLT) count, leukocytes (WBC), hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined [8](Armour et al., 1965).

Biochemical parameters:

The blood without anticoagulant was allowed to clot before centrifugation to obtain serum, which was utilized for the assessment of blood urea nitrogen (BUN), creatinine (CRE), bilirubin, blood glucose (BG), Serum glutamic-oxalo acetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Alkaline phosphatase (ALP) and lipid profile using commercial kits.

Histological studies:

The removed liver, kidney, spleen and heart were fixed in 10% neutral formalin. The histological sections (5µm) of tissues were assessed by haematoxylin and eosin staining method.

Statistical analysis: The statistical data was expressed as mean ±SEM. Parametric data which include all the biochemical parameters were analysed using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests using software Graph pad prism5. The difference between the groups were considered as significant when $p<0.05$.

RESULTS

Acute toxicity studies: The administration of a single oral dose of 2000 mg/kg bd. wt of *Stereospermum suaveolens* induced neither mortality nor treatment-related signs of toxicity in animals throughout the observation period of 14 days. Also, no indications of morbidity were observed.

Sub-acute toxicity studies: Daily oral administration of ethanol extract of roots of *Stereospermum suaveolens* for 28 consecutive days did not induce any obvious symptom of toxicity in rat at both dose levels of 200 and 400mg/kg b. w. No lethality, differences in general behavior, food and water consumption was recorded during the 28 days of extract administration.

Effect of extract on body weight: The change in body weight of all rats in treatment and control groups were similar. The results showed that the extract elicited no significant change in body weight compared to control.

Effect of extract on organ weight: The organ weights were not altered significantly in extract treated groups compared with control (Table 1).

Table 1: Effect of EESS on body weight and organ weight

Group	Body weight gain (g)	Kidney (g)	Liver (g)	Heart (g)	Spleen (g)
I	27.83±1.04	0.73±0.23	6.11±0.07	0.82±0.02	0.85±0.01
II	26.67±0.95	0.76±0.02	6.25±0.21	0.83±0.03	0.86±0.01
III	29.37±0.88	0.75±0.02	5.98±0.09	0.79±0.03	0.84±0.02

Each value represents the Mean ± S.E.M from 6 animals in each group. P>0.05 when compared to control.

Effect of extract on hematological parameters: All the tested hematological parameters were within normal limits compared to control group. No toxicologically significant differences between treated animals with the plant extract and control were found (Table 2).

ISSN: 0975-8585

Table 2: Effect of EESS on haematological parameters

Group	Total RBC (10^6/µL)	Haemoglobin (g/dl)	Platelet count (10^3/µL)	WBC (10^3/µL)	PCV (L/L)	MCV (fL)	MCH (pg)	MCHC (g/dl)
I	7.99±0.26	14.55±0.53	6.90±0.36	10.22±0.75	47.88±1.65	62.02±1.12	18.58±0.47	30.32±0.39
II	8.17±0.33	15.07±0.33	6.69±0.37	9.48±0.24	50.22±0.84	64.68±2.02	19.60±0.29	29.55±0.44
III	8.46±0.18	15.44±0.37	8.07±0.34	10.36±0.43	51.30±1.43	63.68±1.28	19.07±0.64	30.98±0.42

Each value represents the Mean ± S.E.M from 6 animals in each group. P>0.05 when compared to control.

Effect of extract on biochemical parameters: Oral administration of the plant extract at a dose of 200 and 400 mg/kg b.w. did not cause significant changes in serum biochemical parameters such as creatinine, BUN, bilirubin, blood glucose, SGOT, SGPT, ALP, total cholesterol, triglycerides, LDL and VLDL levels when compared to control group (Table 3 and Table 4).

Table 3: Effect of EESS on biochemical parameters of liver and kidney

Group	CRE (mg/dl)	BUN (mg/dL)	Bilirubin (mg/dl)	BG (mg/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
I	0.83±0.05	11.63±0.57	0.77±0.02	68.33±0.84	44.67±2.40	38.50±2.47	85.33±2.73
II	0.86±0.03	11.23±0.47	0.70±0.07	66.67±1.92	37.50±1.86	27.67±1.17	84.50±2.93
III	0.81±0.04	11.47±0.56	0.73±0.07	65.33±2.47	38.83±0.98	28.50±0.99	80.67±4.09

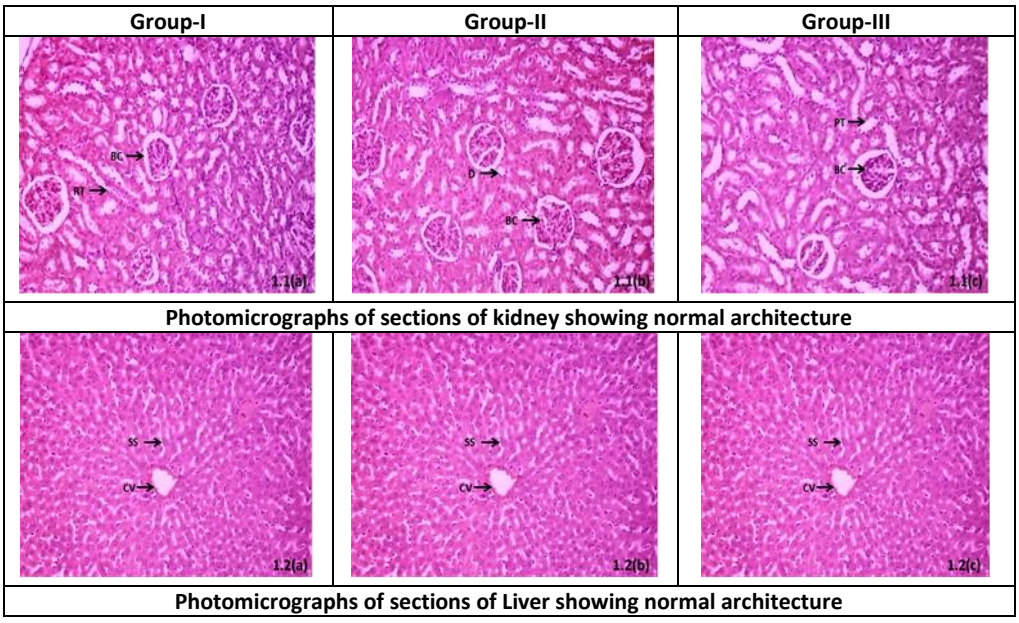
Each value represents the Mean ± S.E.M from 6 animals in each group. P>0.05 when compared to control.

Table : 4 Effect of EESS on lipid profile:

Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	60.17±0.83	52.17±2.60	43.83±0.47	5.89±0.67	10.43±0.52
II	60.33±0.95	55.33±1.93	43.33±0.66	5.97±0.92	11.07±0.39
III	59.67±1.28	52.33±2.50	43.00±0.57	5.87±0.81	10.47±0.51

Each value represents the Mean ± S.E.M from 6 animals in each group. P>0.05 when compared to control.

Histological studies: On histological examination of kidney, liver, heart and spleen it was found that in animals treated with extract there was no observable alteration in histo-architecture (Figure 1).



ISSN: 0975-8585

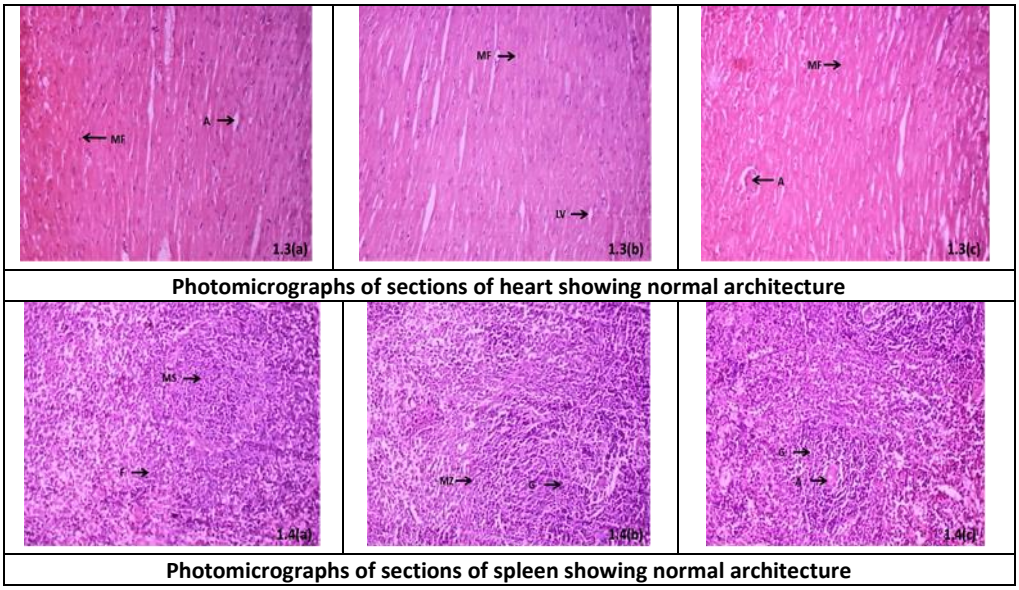


Figure 1: Photomicrographs of rat vital organs. BC-Bowman's capsule, D-Distal tubule, RT-Renal tubule, PT-Proximal tubule, SS-Sinusoids space, CV-Central vein, A- Arteriole, MF- Myocardial fibers, LV-Lymphatic vessel, MS-Marginal sinus, MZ-Marginal zone, F- Follicle, G- Germinal center.

DISCUSSION

Lack of pharmacological and toxicological evaluation substantially restricts their therapeutic application in ethno-medicine and ethno-veterinary medicine in this developing world. Accordingly, laying down of toxicological studies are paramount in the development of botanical products to safe and efficacious drugs [9] (Wu et al., 2000). In fact, a toxicological evaluation should be performed in various experimental animals to determine the potential adverse effects and provide guidelines for the selection of a dose that is "safe" for humans [10](Rhiouania et al., 2008).

In acute toxicity study, *Stereospermum suaveolens* at 2000mg/kg was administered orally as recommended by the OECD 423 guidelines. No lethality and signs of toxicity were recorded and, therefore, the oral toxicity of this fraction can be classified in category 5 (the lethal acute toxicity is greater than 2000mg/kg) according to the Globally Harmonized Classification System of OECD.

The sub-acute toxicity refers to the cumulative detrimental effects resulting from a substance administration, preferably oral, in a limited period of time (15–30 days) [11](Steuer et al., 2013). A 28-day sub-acute toxicity testing using repeated doses is widely considered satisfactory to assess any possible health hazards. Hence in the current study this was employed.

Body weight changes and growth rate in prolonged toxicity studies are important indicators of adverse effects of drugs and chemicals on laboratory animals [12](Amenya et al., 2011). The normal increase in body weight in drug administered groups compared with control usually indicate absence of toxicity as decrease in body weight is an index of toxic effect of a compound.

Haematopoietic system is one of the most susceptible targets of toxic compounds, especially in the bone marrow where the production of red blood cell occurs [10][Rhiouania et al., 2008]. Sub-acute administration of the ethanol extracts of *S. Suaveolens* did not cause significant changes ($P > 0.05$) in the haematological profile of rats that received the entire test doses when compared with control, suggesting that *S. suaveolens* may not be toxic to the blood system.

Biochemical parameters such as serum creatinine, BUN, SGOT, SGPT, ALP and bilirubin were evaluated in current study because they are the first symptoms of toxicity that organs show when exposed to possible toxic substances [13] (Muhammad et al., 2011). Kidney functions were evaluated by means of BUN and SC and an increase in these markers is a good indicator of negative impact in kidney functions

[14,15](Atsamo et al., 2011; Ezeja et al., 2014). Liver functions were assessed by determining SGOT, SGPT, ALP and bilirubin and an elevation in these markers indicates hepatic damge [16](Chavda et al., 2010). However, based on the statistical analyses of these renal biochemical parameters and liver functional markers, no significant difference was observed in any of the parameters in the kidneys and livers of rats of treated with the EESS, suggesting that the plant presents no toxicity to these organs. Further lipid profile was also evaluated in current study. The levels of TC, TG, HDL, LDL and VLDL in extract treated animals remained unchanged which indirectly indicates normal liver functioning.

Alteration in organ weight may be as a result of organ damage [17](Busari et al., 2015). In this study, the weight of the vital organs (kidney, liver, heart and spleen) after the treatment period indicated no significant obvious change. The result is an indication that *S. Suaveolens* may not elicit any deleterious effect on the weight of kidney, liver, heart and spleen and the result is in consonance with earlier findings [18](Olorunnisola et al., 2012). Moreover histological observations of major organs also evident the absence of any toxicological effects in extract treated groups.

CONCLUSION

On the basis of findings of the present study, it can be concluded that ethanol extract of roots of *Stereospermum suaveolens* was practically non toxic in rats after oral administration. It did not produce any behavioral, hematological, biochemical or histopathological symptoms of toxicity after 28 days of continuous oral administration (at dose levels 200 and 400mg/kg b.w), which supports the use of roots of *Stereospermum suaveolens* in traditional systems of medicine.

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