

Study of Acute and Sub Chronic Toxicity of *Spathodea campanulata* P Beav Leaf

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Abstract. The safety use of the ethanol extract of *Spathodea campanulata* leaf was evaluated using Swiss albino mice. Increasing dose of 100-250mg/kg of the extracts was administered to the mice orally for acute toxicity where toxic symptoms and mortality were observed on the mice for 48h. 200mg/kg of the extract was administered weekly for six weeks to the mice for sub chronic toxicity through haematological, biochemical antioxidant and histological studies. Results obtained in the haematological profile, biochemical, hepatorenal analyses, antioxidant activities and histological status of the mice liver suggests non toxicity of the extract. Minimum dose of 250mg/kg was found acceptable as a safe dose in acute toxicity. The sub chronic toxicity as well could not suggest signal(s) of toxicity in the mice. Though some alterations were found in the parameters examined, it was minimal and could be as a result of the stress during the toxicity assay.

Keywords: Plant, Ethanol extract, Toxicity, Acute, Sub chronic

1. Introduction

Traditional medicine is basically with the use of medicinal plants in decoction and concoction preparations. Because of their values in treatment of diseases by choice in many developing countries, their world wide use is lagging because of the lack of scientific bases to support their safety use. The affordability, reliability, availability and low toxicity of medicinal plants in therapeutic use has made them popular and acceptable by all religions, for implementation in medical health care all over the world (Akharaiyi, 2011). The modern pharmaceutical industries itself still relies largely on the diversity of secondary metabolites in plants of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Mallikharjuna et al., 2007). The search for plants with antimicrobials has gained increasing importance in recent years due to the development of antimicrobial drug resistance and often the occurrence of undesirable side effect of some antibiotics (Davis, 1994, Service, 1995).

Spathodea campanulata P. Beav species popularly known as African tulip tree belong to Bignoniaceae family. This plant has many uses in folk medicine such as the leaves being employed against kidney disease, urethra inflammations and as antidote against animal poisons. The stem preparations are employed against enemas, fungus skin disease, herpes, stomach ache, and diarrhoea (Adriana et al., 2007). Traditionally, the seeds, bark and leaves are used for swollen cheeks, cleaning of new born babies and antimalarial (Anon, 1986), mulluscicidal, antioxidant (Makinde et al., 1998; Akharaiyi et al., 2012). Despite its popularity, in folk medicine, the toxicity profile has not been yet explored. This present study is based on the acute and sub chronic toxicity of the ethanol extract of *S. campanulata* leaf in Swiss albino mice.

2. Methodology

2.1. Collection of Plant and Extract Preparation

Apparently healthy leaf, of *Spathodea campanulata*, were collected from Aule forest in Akure South Local government, Ondo State, Nigeria and was authenticated by Dr. Oyun, M.B, Department of Forestry and Wood Technology, Federal University of Technology, Akure, Nigeria. The specimen voucher number

AF 1506 was deposited for future reference. The plant leaves were dried for three weeks and then ground to powder with a mechanical grinder. The powders obtained were extracted with ethanol at room temperature (25+ 2⁰C). The resulting crude extract was filtered with sterile muslin cloth and concentrated using a rotary evaporator RE-52A (Union Laboratories, England). The obtained dried crude extract was contained in plastic container before use.

2.2. Experimental Animals

Apparently healthy Swiss albino mice of between 20 – 23g were used. The animals were contained in a cage and maintained under standard laboratory conditions. They were giving rodent pellets (Vital feeds) and water *ad libitum*. They were acclimatized for 2 weeks and were fasted over night with free access to water prior the experiments. The animals were conducted in compliance with NIH Guide for Care and Use of Laboratory Animals.

2.3. Acute Toxicity

Apparently healthy Swiss albino mice of between 20 – 23g of either sex were starved overnight and were divided into six groups of ten per group. Group I – V mice were orally fed with EESC in increasing dose levels of 100, 150, 200, and 250mg/kg b wt while group VI receive 1% tween 80 and served as (control experiment). The animals were observed continuously for 48hr for any gross change in behavioural, neurological, autonomic profiles and mortality in each group. One- fifth of the maximum safe dose of the extract tested for acute toxicity was selected for the sub chronic toxicity assay.

2.4. Sub Chronic Toxicity Study

Sixteen healthy mice were divided into two groups of eight mice in each. Group I (normal control) mice received 1% Tween 80 solution (1.0 ml, p.o.) and Group II mice received EESC (200mg/kg) for every 48 h for six weeks. The mice were weighed every three days and food and water intake were monitored daily. After the last dose at the end of the experiment, the mice were starved for 18h and sacrificed. The blood was collected into heparinised tube for haematological studies and non-heparinised centrifuge tube for biochemical assay. Liver was collected for *in vivo* evaluation of antioxidant status and histopathology.

2.5. Haematological Studies

Neubauer haemocytometer was used for the enumeration of RBC and WBC. Haemoglobin estimation was by using Sahli's Hemoglobinometer by standard procedures from the blood obtained intracardially with the criteria of D' Armour et al., 1965.

2.6. Biochemical Estimation

The effect of EESC treatment on the biochemical parameters of the experimental mice were evaluated by the estimation of serum biochemical enzymes such as serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities by the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) activities was by the method of Kind and King (1954), total bilirubin (Malley and Evelyn, 1937), total protein (Weichselbaum, 1946), urea (Krieg et al., 1986), uric acid (Caraway, 1963), creatinine (Larsen et al., 1971), glucose, total cholesterol, triglyceride, HDL and LDL cholesterol (Wasan et al., 2001). All the analyses were performed by standard enzymatic methods using commercially available kit from Randox Laboratories Ltd, UK.

2.7. In vivo Antioxidant Assay

The antioxidant assay was performed with the liver tissues of the experimental mice and evaluation of the antioxidant status was carried out by measuring the level of lipid per oxidation (Ohkawa, 1979) and the amount of enzymatic (Catalase: CAT) and non enzymatic antioxidant system (reduced glutathione: GSH) by the methods of Luck (1963) and Ellman, (1959) respectively.

2.8. Histological Studies

After sacrificing the mice, parts of liver tissues were collected for the histological studies. The tissues were washed in normal saline and fixed immediately in 4% formalin for about 2h, dehydrated with grades of

alcohol, and embedded in paraffin, cut into 5-7 μ m thick sections and stained with haematoxylin-eosin dye for photo microscopic observation.

2.9. Statistical Analysis

Values were presented as mean \pm S.E.M. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test using SPSS software. $P < 0.01$ was considered as statistically significant.

3. Results and Discussion

On the administration of *S.campanulata* leaf ethanol extract to the mice, significant difference were not found in the erythrocyte and leucocytes values of both the treated and control mice. In which case, the administration of *S. campanulata* leaf ethanol extract for a period of 48h cannot induce anaemia. Though minor irregularities were observed mainly in the WBC, this could be as a result of the mice response to foreign bodies associated with the chronic toxicity during the experiment (Kelly, 1977). The toxicity assay did not result into abnormality and mortality of the tested mice for the period of 48h monitored. With this result where no adverse effect was seen in the administration of EESC, it implies that the medium lethal dose (LD₅₀) is probably higher than 250mg/kg in both the male and female mice.

However, the normal levels of hepatic biomarker enzymes (SGOT, ALP and SGPT) total; bilirubin and protein in serum and the control mice values of renal biochemical parameters (Urea, uric acid, and creatinine) suggests that sub chronic assay with EESC does not possess a significant effect on hepato-renal functions of the mice (Table 1). SGOT, ALP and SGPT in this study were not high compared to the control group results to report sign of liver damage (Table 2). This observation is in agreement with Saha et al., (2010). Hence the bilirubin level was not increased; jaundice could not be resulted in the intake of this extract. However, the lipid profile and blood sugar level of both the control and the treated mice remain normal as no significant alteration to record as being adverse effect in the mice (Table3). The extract seemed to serve as protective means on the lipid profile of the mice which was clearly indicated by the values obtained from the treated mice in comparison to the control mice values in LPL and HDL cholesterol. Plates 1 and 2 shows the histological status of the liver tissues of both the treated and control mice where normal cellular architecture with prominent central vein was shown which indicates, that the extract cannot cause damage to livers if used for therapeutic purpose. This becomes important because liver is the primary organ for detoxification. The tested mice endogenous antioxidant status after chronic assay of the extract was found adequate on the endogenous antioxidant system which may be beneficial in case of oxidative stress of any disease condition. The criticism in traditional medicine is the lack of scientific evaluations to justify its tremendous impact in its use as a safe drug. Studies into the toxicity of traditional medicine hence are natural products would determine the efficacy and safety use. The results obtained however suggest this plant for apparent use in therapeutic purpose.

4. References

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Table 1: Effect of Ethanol extracts of *Spathodea campanuluta* leaf on haematological parameters of control and tested mice

Groups	Haemoglobin (g %)	RBCmillion/cu. mm	WBC thousand/cu.mm
Control (1% Tween 80)	12.11 ± 1.02	6.57 ± 0.85	3.2 ± 0.26
EESC (200mg/kg)	10.75 ± 1.10	6.76 ± 0.50	4. 00 ± 0.77

Table 2: Effect of Ethanol extract of *Spathodea campanuluta* leaf on biochemical parameters of hepatorenal function in control and tested mice

Group	SGOT (IU/dl)	SGPT (IU/dl)	SALP (IU/dl)	Bilirubin (Mg/dl)	Total Protein (g/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Control (1% Tween 80)	54.50±1.60	43.20±4.01	87.25±2.88	1.08±0.10	7.15±0.45	39.15±3.50	6.05±0.60	1.0±0.30
EESC 200mg/kg	60.02±2.87	48.45±3.67	95.04±3.67	1.15±0.10	6.75±0.70	36.75±2.98	52.69±0.9	1.62±0.74

Table 3: Effect of Ethanol extracts of *Spathodea campanuluta* leaf on lipid and glucose level in control and tested mice (mg/dl)

Groups	Triglyceride	Total cholesterol	HDL	LDL	Glucose
Control (1%Tween 80)	86.60±3.20	118.40±2.40	77.65±2.60	25.75±6.97	83.55±3.05
EESC 200mg/kg	94.11±4.4	126.03±6.05	78.90±2.65	29.02±3.60	89.04±3.45

Table 4: Effect of Ethanol extract of *Spathodea campanuluta* leaf on antioxidant system in control and tested mice (mg/dl)

Group	LPOnM/mg wet tissue	GSHµg/mg wet tissue	CAT (µM of H ₂ O ₂ decomposed/min/mg wet tissue)
Control (1% Tween 80)	102.23±5.54	36.68±1.52	70.62±5.55
EESC 200mg/kg	108.09±3.05	40.01±2.81	78.03±3.63

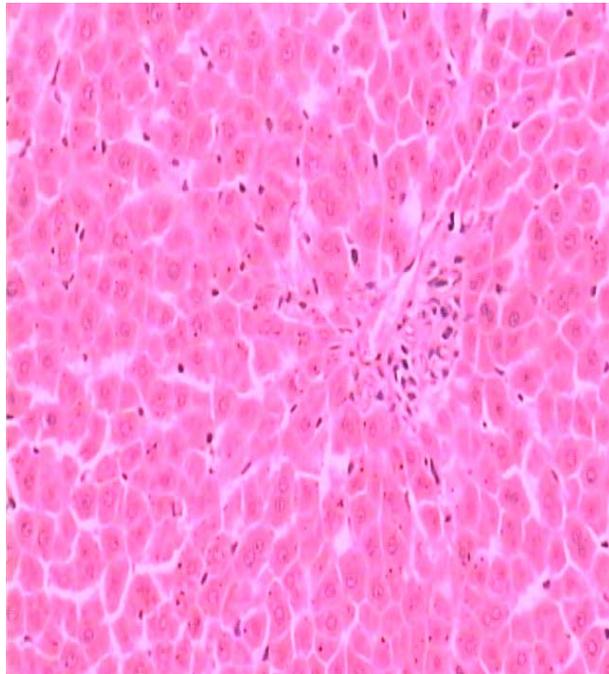


Fig. 1: Control

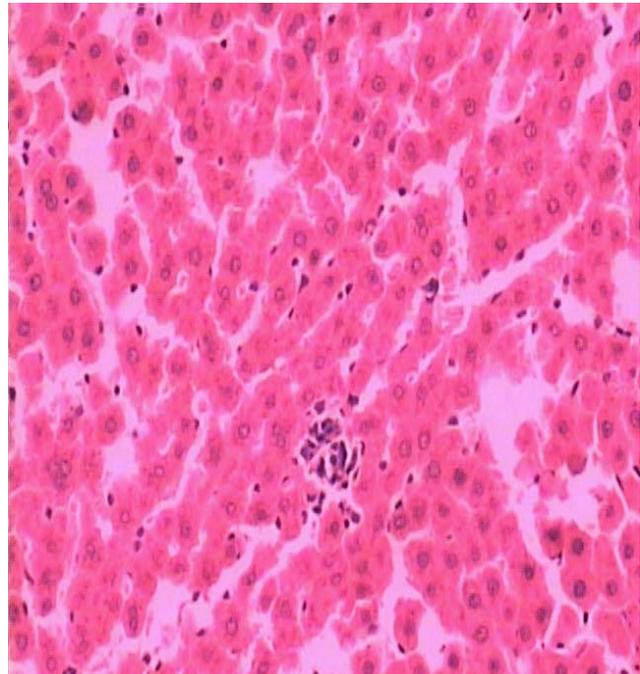


Fig. 2: Treated