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Acute and sub-acute toxicity studies of hydroalcoholic extract from *Acacia suma* (Roxb) (Fabaceae) stem barks

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Abstract

Herbal medicines are popular remedies for diseases used by a vast majority of the world's population. The pharmacological effects of many plants have been studied in various laboratories, whereas there are many limitations regarding the safety and efficacy. Our research group has previously investigated the phytochemical and pharmacological properties of this plant. The present study was carried out to evaluated acute and subacute toxicity of hydroalcoholic extract from stem bark of Acacia suma (Roxb.) var. Acacia polyacantha (Family- Fabaceae). Acute toxicity study was evaluated on male Swiss albino mice (20-25g) and Wistar albino rats (150-200g) were assigned for sub-acute toxicity, after ingestions of the extract during one day (acute model) and during 15 days (subacute model). The acute toxicity studies were conducted as per the OECD guidelines 423, where the limit test dose of 3000 mg/kg b.wt., p.o., used. Results showed that the LD50 of the extract is higher than 3000 mg/kg b.wt., and there was no mortality was observed at 3000 mg/kg b.wt., p.o., dose except with prominent diuresis and purgation, these toxic sign are probably due to the saponins content of the extract; so, testing of the hydroalcoholic extract of Acacia suma stem bark at higher dose was practically non-toxic. In sub-acute toxicity study, the extract treated groups (300 and 600 mg/kg b.wt., p.o.) did not show any significant changes in body weight when compared to the control group. The weight of the liver, kidney and pancreas were found to be unaltered in the experimental groups compared with the control group. The haematological and biochemical parameters (hepatic and renal function tests) did not show any significant changes in the sample treated groups when compared with the control group animals.

Keywords: *Acacia suma*, Acute, Sub-Acute Toxicity Study, Haematological, Biochemical Parameters.

Introduction

The use of herbal heritage has become a part of general health care by the tribes since time immemorial. The use of modern medicines of synthetic origin imparting dramatic results in a short span in the therapeutic field laid several side effects upon long term use. Traditional medicaments, chiefly obtained from plants have played a vital role in sustaining disease free human existence on this planet [1]. It is rather difficult to date back the origin of these medicaments as a means of therapy. In spite of overwhelming influence of modern medicine and tremendous advances made on the production of synthetic drugs, traditional medicaments designated now a days as herbal drugs in different places in literature, have retained their place in therapy. Their effectiveness, low cost and comparative freedom from serious toxic effects makes these medicaments not only popular but also an acceptable mode of treating diseases even in modern times [2].

Acacia suma (Roxb.) var. *Acacia polyacantha* (Family- Fabaceae) is a medium sized erect tree; trunk with fissured bark and knobby persistent prickles found in the greater part of India and costal districts of Orissa [3, 4]. The dried stem bark is used as folklore medicine in the treatment of anemia, uterine complaints and reported to possess astringent, analgesic, anti-inflammatory and antiseptic properties [5]. The seeds are reported to have hypoglycaemic effect and bark is reported to be used as blood purifier, possesses anti-cancer and astringent properties [6], Similarly the various extracts of stem bark is also reported for hypoglycaemic activity in normoglycaemic and alloxan induced hyperglycaemic rats [7] and according to Mondal *et al.* [8] reported the diuretic and laxative effects of aqueous extract of *A. suma* barks at 200 and 400 mg/kg, b.wt., p.o. Acacia polyphenol also inhibited the lipase and glucosidase activities [9]. Indole alkaloid namely tryptamine, N-N- dimethyl is isolated from leaves [10]. Presences of proanthocyanidin, quercetin [11] and 5,4'-dihydroxy-7,3'- dimemethoxyflavone-3-0-D galactopyranoside in the stem bark have been reported earlier [12].

Due to the widespread use of this plant by the rural communities to treat several diseases, the objective of the present study is to investigate acute and subacute toxicity of hydroalcoholic extract from stem bark of *A. suma* in experimental animal model. The changes in selected biochemical and hematological parameters were also determined.

Material and Methods

Plant material

The stem bark were collected from young matured plant from rural belt of Visakhapatnam and authenticated by the taxonomists of Botanical Survey of India, Shibpur, Howrah. A voucher specimen [Sp. No: CNH/I-I/(17)/20011/Tech.II/28] has been kept in our research laboratory for further reference. The material was washed, shade dried and powdered.

Preparation of the extract

The powdered plant materials were macerated with 70% ethanol (w/w) at room temperature for one week and then filtered and concentrated in rotary evaporator (*Evator*, Media Instrument Mfg. Co., Mumbai, India) under reduced pressure to obtain a dark greenish-brown hydroalcoholic residue (10.76%). Preliminary phytochemical studies were performed on the extract using standard procedures [13-15].

Animals

Animals used in this study were male Swiss albino mice (20-25 g) and male Wistar rats of (150-210 g). The animals were housed for at least one week in the laboratory animal room prior to testing in standard polypropylene cages at room temperature of 34 ± 2^0 C and at 60-65% relative humidity. Food (fed with commercially pellet diet supplied by M/s Hindustan Lever Ltd., Mumbai) and water were given *ad libitum* unless otherwise specified. All experimental protocols (Protocol No.: IAEC/GIP-1287/M Pharm/IP/SM-SA/2011-12) were approved by the Institutional Animal Ethics committee of GITAM Institute of Pharmacy, Visakhapatnam, Andhra Pradesh, India. The experiments were designed in different groups containing six animals in each.

Acute toxicity study

The acute toxicity studies were conducted as per the OECD guidelines 423 [16], where the limit test dose of 3000 mg/kg b.wt., p.o., used. The test was carried out as suggested by Ganapaty *et al.*, [17] and Akhand *et al.*, [18]. The control group received only vehicle (3 ml/kg b.wt., p.o.). The other groups separately received 100, 2000 and 3000 mg/kg b.wt., p.o., of the test extract respectively in a similar manner. Immediately after dosing, the animals were closely observed for the initial 4 hrs after the administration and then once daily during the following days. The behavioural changes closely observed for were hyperactivity, ataxia, convulsion, salivation, tremors, diarrhoea, lethargy, sleep and coma. They were then kept under observation up to 14 days after drug administration to find out the mortality if any. One-fifth and one-tenth of the maximum tolerated dose of the hydroalcoholic extractof *A. suma* stem bark tested for acute toxicity (3000 mg/ kg b.wt., p.o.) was selected for the subacute toxicity study (300 and 600 mg/ kg b.wt., p.o.).

Sub-acute toxicity study

The Sub-acute toxicity was carried out as suggested by Halim *et al.*, [19] and Das *et al.*, [20]. Wistar rats were randomly assigned into three groups (6 per group). Group I received distilled water (3 ml/ kg b.wt., p.o.) through oral route for 14 days and Groups II and III received hydroalcoholic extract of *A. suma* stem bark at dose of 300 and 600 mg/ kg b.wt., p.o., once daily for 14 days. All rats were observed daily for physiological and behavioural changes. Body weight, food intake and water intake were monitored. The animals were sacrificed on day 15, blood samples were collected for hematological parameters [21] like hemoglobin percentage (Hb%), red blood cell count (RBC), white blood cell count (WBC), differential count (DC) [neutrophils (N), lymphocytes (L), basophils (B)] and biochemical parameters [22, 23] like blood urera, Aspartate transaminase (AST), alanine transaminase (ALT), serum creatinine and alkaline phosphatase (ALP) were assed using commercial kits., supplied by Span Diagnostics Ltd., Surat, India. Histopathological investigation of the liver, kidney and pancreas was done according to the describe method [24, 25].

Statistical Analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's-t test. A *P*-value < 0.05 was considered to be significant. All the values were expressed as mean ± SEM.

Results and Discussion

Preliminary phytochemical tests revealed the presence of saponins, carbohydrates, flavonoids, tannins and phenolic compounds in hydroalcoholic extract of *A. suma* stem bark.

In acute toxicity study, when orally administered hydroalcoholic extract to mice in graded doses from 100, 2000 and 3000 mg/ kg b.wt., p.o., the studies revealed that extracts induced diuresis and purgation at all tested doses, these toxic sign are probably due to the saponins content of the extract [26, 27]. However, there was no mortality or any significant changes in general behavior, breathing, sensory nervous system responses and cutaneous effects during the study. So according to Organisation for Economic Cooperation and Development (OECD-423) guidelines for acute oral toxicity, LD_{50} dose of 3000 mg/kg and above is categorized as unclassified and hence the extract was said to be practically non-toxic and found to be safe. As 3000 mg/kg of body weight was well tolerated by the animals without any behavioural changes hence, one-fifth and one-tenth of the maximum tolerated dose for acute toxicity

(3000 mg/ kg b.wt., p.o.) was selected for the subacute toxicity study (300 and 600 mg/ kg b.wt., p.o.).

In the sub-acute toxicity study, hydroalcoholic extract of *A. suma* stem bark at doses of 300 and 600 mg/ kg b.wt., p.o., did not show any significant changes in the body weight increment (Table 1), indicating that it did not have any adverse effects on body weight, which is used to assess the response to therapy of drugs [28] and to indicate the adverse effects of a drugs [29]. The weight of the liver, kidney and pancreas (Table 2) were found to be unaltered in the experimental groups compared with the control group, indicating that plants extracts were non toxic in these vital organs. The haematological and biochemical parameters (hepatic and renal function tests) summarized in Tables 3, 4 and 5. It was found that there were no significant changes in any liver function parameters (Table 3), such as Total bilirubin, Conjugated bilirubin, Total protein, Albumin, Total cholesterol, Triglyceride, ALT, AST and ALP, compared to the control group. Increase in these parameters would have indicates hepatocytes damage [30]. The normal levels of blood urea and serum creatinine (Table 4) indicated that the test extract of *A. suma* barks, did not interfere with renal function and that renal integrity was preserved [31]. Also, there were no significant changes in various haematological parameters (Table 5) such Hb, total count (TC) of RBC and WBC, differential count (DC) of WBC and platelet count compared to the control group, which indicates that test extracts may not be toxic and do not affect circulating red cells, hematopoiesis or leucopoiesis.

The results of the histopathology study revealed no sign and symptoms of degeneration on the isolated organs under study.

Multiple sections of the liver studied showed preservation of normal lobular architecture. Hepatocytes appear normal and arranged in single cell cords radiating away from central vein. No sign of non specific lobular hepatitis was observed at the tested dose levels of the extracts. There was no evidence of bile stasis, granuloma, dysplasia or malignancy (Figure 1), furthermore multiple sections studied from renal biopsy show normal size and shape of glomeruli, tubules, interstitium and blood vessels. There is no evidence of acute tubular necrosis or glomerular changes (Figure 2) and in multiple sections of the pancreas studied also showed preservation of normal architecture. There was no evidence of histopathological lesions, granuloma, dysplasia or malignancy (Figure 3).

Conclusion

Present observation indicate that hydroalcoholic extract of *Acacia suma* stem barks have broad safety margin in experimental animals commonly used in *In-vivo* experimental and preclinical pharmacological studies. The study establishes the reliable safety of hydroalcoholic extract of *Acacia suma* stem barks in Swiss albino mice and Wistar albino rats offering no obvious toxicity.

Table 1: Effects on body weight of rats in 15 days treatment of hydroalcoholic extract of *A. suma* stem bark.

Group	Treatment	Dose	Average body weight (g)	
			0 day	15^{sh} day
I	Control	3 ml/kg	165.41±1.1	163.33±1.53
II	Hydroalcoholic extract of *A. suma*	300mg/kg	168.11±1.27	169.9±0.99
III	Hydroalcoholic extract of *A. suma*	600 mg/kg	158.3±1.49	160.76±1.24

Values are expressed as mean ±S.E. from six observations.

Table 2: Effects of 15 days oral administration of hydroalcoholic extract of *A. suma* stem bark on organ weights in rats.

Parameter	Control (3 ml/kg, p.o.)	Hydroalcoholic extract of *A. suma* (300mg/kg, p.o.)	Hydroalcoholic extract of *A. suma* (600mg/kg, p.o.)
Total bilirubin (µmol/l)	12.9±1.5	13.9±1.5	14.05±1.8
Conjugated bilirubin (µmol/l)	3.55±0.96	4.06±1.1	4.46±1.2
Total protein (g/l)	72.73±5.53	71.03±5.36	72.23±7.48
Albumin (g/l)	37.98±2.69	36.35±2.54	38.11±2.44
Total cholesterol (mg/dL)	65.13±5.59	66.18±6.63	67.48±6.95
Triglyceride (mg/dL)	84.56±5.97	82.13±6.38	86.73±6.76
ALT (IU/l)	25.65±3.01	24.91±3.3	25.75±2.96
AST (IU/l)	10.76±1.65	11.16±1.85	12.06±1.91
ALP (IU/l)	116.38±6.36	117.53±7.74	117.45±9.44

Values are expressed as mean ±S.E. from six observations.

Table 3: Biochemical parameters for Liver function tests on rats of hydroalcoholic extract of *A. suma* stem bark

Group	Treatment	Dose	Organ weight (g/100g body weight)		
			Liver	Kidney	Pancreas
I	Control	3 ml/kg	3.04±0.16	0.76±0.02	0.35±0.04
II	Hydroalcoholic extract of *A. suma*	300mg/kg	2.91±0.11	0.76±0.03	0.34±0.01
III	Hydroalcoholic extract of *A. suma*	600 mg/kg	3.13±0.11	0.73±0.03	0.37±0.04

Values are expressed as mean ±S.E. from six observations.

Results are expressed as mean±S.E. from six observations. ALT: Alanine transaminase also called Serum Glutamic Pyruvate Transaminase (SGPT);AST: Aspartate transaminase also called Serum Glutamic Oxaloacetic Transaminase (SGPT); ALP : Alkaline phosphatase.

Table 4: Effect of 14 days administration of hydroalcoholic extract of *A. suma* stem bark on kidney function in rats.

Values are expressed as mean ±S.E. from six observations.

Group	Treatment	Dose	Blood Urea (mg %)	Serum Creatinine (mg %)
I	Control	3 ml/kg	19.96±0.69	1.55±0.27
II	Hydroalcoholic extract of *A. suma*	300mg/kg	20.48±0.91	1.57±0.24
III	Hydroalcoholic extract of *A. suma*	600 mg/kg	21.33±1.01	1.49±0.15

Table 5: Effect of intake of hydroalcoholic extract of *A. suma* stem bark on some haematological parameters.

Parameter	Control (3 ml/kg, p.o.)	Hydroalcoholic extract of *A. suma* (300mg/kg, p.o.)	Hydroalcoholic extract of *A. suma* (600mg/kg, p.o.)
Hb (%)	13.04±0.6	13.95±0.9	12.81±.92
RBC (X 10^{12}/l)	9.25±0.83	9.13±0.79	10.29±0.94
WBC (X 10^{9}/l)	13.38±0.59	13.45±0.74	14.56±0.63
Platelet (X 10^{9}/l)	555.33±40.95	571.83±41.22	584.5±4.18
Neutrophils (%)	47.18±3.25	47.94±4.14	48.25±4.18
Lymphocytes (%)	57.28±5.74	56.08±6.49	57.06±6.59
Eosinophils (%)	1.46±0.16	1.50±0.17	1.28±0.15
Monocytes (%)	1.08±0.11	1.01±0.09	1±0.12
Basophils (%)	00±00	00±00	00±00

Values are expressed as mean ±S.E. from six observations

Results are expressed as mean + S.E. from six observations. Hb: Haemoglobin; RBC: Red Blood Cells; WBC: White Blood Cells.

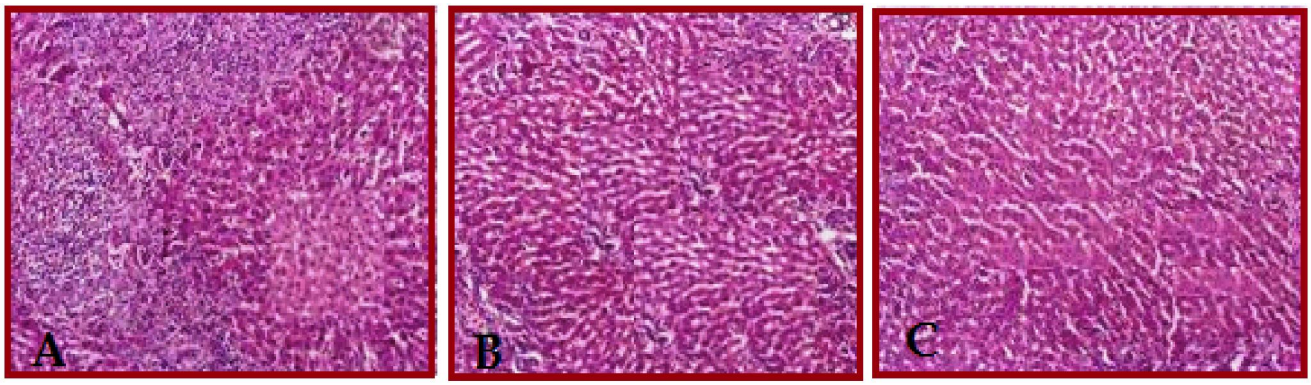

Figure 1: Histopathological image of rat's liver; (A) Liver of control rat (3 ml/kg, p.o.); (B) Liver of rat receive hydroalcoholic extract of A. suma stem bark (300 mg/ kg, p.o.); (C) Liver of rat receive hydroalcoholic extract of A. suma stem bark (600 mg/ kg, p.o.)

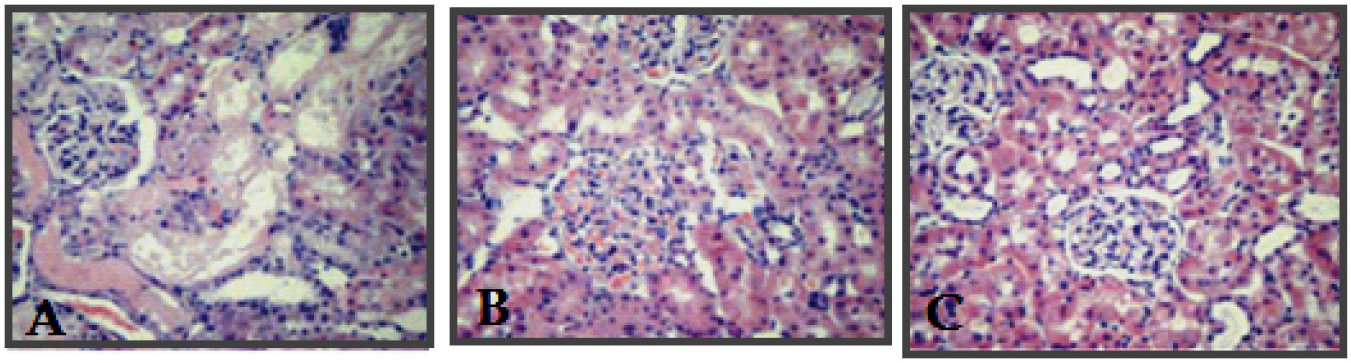

Figure 2: Histopathological image of rat's kidney (A) Kidney of control rat (3 ml/kg, p.o.); (B) Kidney of rat receive hydroalcoholic extract of A. suma stem bark (300 mg/ kg, p.o.); (C) Kidney of rat receive hydroalcoholic extract of A. suma stem bark (600 mg/ kg, p.o.)

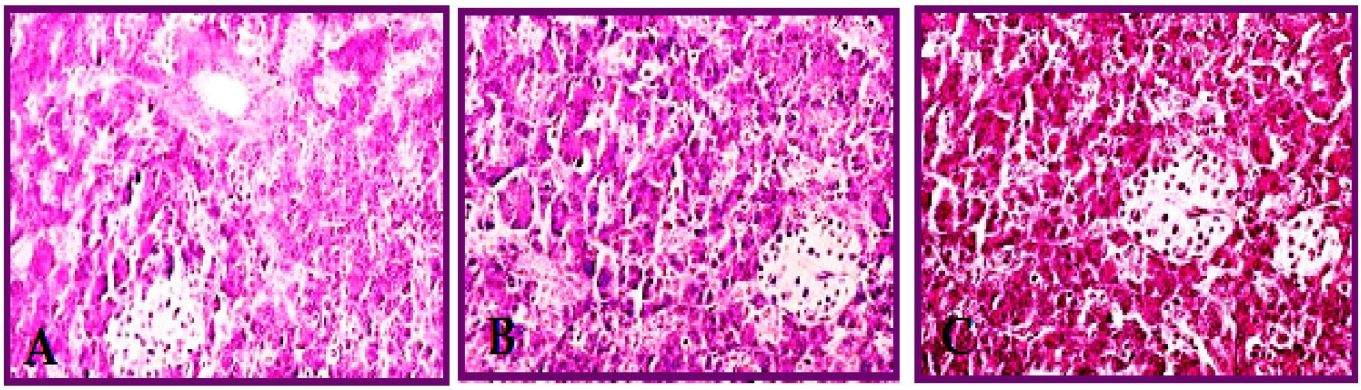

Figure 3: Histopathological image of rat's pancreas : A) Pancreas of control rat (3 ml/kg, p.o.); (B) Pancreas of rat receive hydroalcoholic extract of A. suma stem bark (300 mg/ kg, p.o.); (C) Pancreas of rat receive hydroalcoholic extract of A. suma stem bark (600 mg/ kg, p.o.)

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