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ACACIA VISCO-INVESTIGATIONS OF ACUTE AND CHRONIC TOXICITY

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ABSTRACT

Acacia visco is a perennial tree found at higher elevations in northern Argentina, Bolivia, Chile and Peru. It has also been introduced to Africa. Common names for it include arca, visco, viscote, viscote blanco and viscote negro. It grows 6–25m tall and it has fragrant yellow flowers in the Spring. In Bolivia is found at an altitude of 1500–3000m. It has light to dark reddish brown twigs and small white flowers. It is cultivated for use in cabinetmaking. This study focuses on establishing the toxicity profile of the plant extract. The plant extract will be tested for acute and chronic toxicity as per OECD guidelines. The present investigation demonstrates that at doses consumed in the traditional medicine, the ethanol extract of Acacia visco L. may be considered as relatively safe, as it did not cause either any lethality or changes of in the general behavior in both the acute and chronic toxicity studies in rats.

Keywords: *Acacia visco L,* acute toxicity, chronic toxicity, lethal dose.

INTRODUCTION

Acacia visco is a perennial tree found at higher elevations in northern Argentina, Bolivia, Chile and Peru. It has also been introduced to Africa. Common names for it include arca, visco, viscote, viscoteblanco and viscote negro. It grows 6–25m tall and it has fragrant yellow flowers in the Spring. In Bolivia is found at an altitude of 1500–3000m. It has light to dark reddish brown twigs and small white flowers. It is cultivated for use in cabinetmaking [1]. Methanol extract of Acacia visco has been shown to have short-term and long-term anti-inflammatory effects in lab rats.[6] Among the class of compounds characterized from A. visco leaves, the triterpenoid lupeol, α-amyrin and β-amyrin may be mainly responsible for the pharmacological activities. This study focusses on establishing the toxicity profile of the plant extract. The plant extract will be tested for acute and chronic toxicity as per OECD guidelines.

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MATERIALS AND METHODS

Plant Material

The Plant material of *Acacia visco L.* used for investigation was collected from a local store and were duly authenticated and the herbarium sample is deposited in the college library.

Extraction

The plant material of *Acacia visco L.* was dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (100gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of ethanolic extract of *Acacia visco L.* was found to be 18.2 % w/w.

Animals used

Albino wistar rats (150-230g) of either sex were

obtained from the animal supplier from Bengaluru. All animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute toxicity study

The procedure was followed by using OECD 423 (Acute Toxic Class Method) [2]. The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality or moribund status of the animals and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use number of animals while allowing for acceptable data based scientific conclusion. The method used to defined doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). The starting dose level of EEGS was 2000 mg/kg body weight p.o as most of the crude extracts posses LD 50 value more than 200 mg/kg p.o. Dose volume was administered 0.2ml per 100gm body weight to overnight fasted rats with were *ad libidum*.

Food was withheld for a further 3-4 hours after administration of EEAV and observed for signs for toxicity. The body weight of the rats before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted. Hence, $1/20^{th}$ (100mg/kg), $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study.

Chronic Toxicity study

Design of Treatment

Animals were divided into 5 groups of six rats each.

Group 1: Normal saline (0.9%, NaCl, 5ml/kg, p.o) once in a week for 6 weeks.

Group 2: Vehicle 1% SCMC (5ml/kg, p.o) once in a week for 6 weeks.

Group 3-5: Ethanolic extract of *Acacia visco L.* at the dose of 100, 200 and 400 mg/kg, p.o respectively. Animals from each group were sacrificed at the 6^{th} week, after the last dose. Different haematological and serum biochemical tests were then performed.

Blood and Serum sample collection

Paired blood samples were collected by cervical decapitation from diethyl ether anaesthetized rats into heparinised bottles for haematological studies and clean non-heparinised bottles and allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis.

Hematological estimation

Estimation of Hemoglobin [3], RBC count [4]**,** WBC count [4]**,** different leucocytic count [3]**,** Elongation time [3] and ESR [5] were determined according to the standard procedures.

Serum biochemical estimation

Blood Glucose [6], Serum Bilirubin [7]**,** Serum Gluconate – Oxaloacetate Transaminase (SGOT) [7], Serum Glutamate – Pyruvate Transaminase (SGPT) [7], Serum Alkaline Phosphatase (ALP) [7]**,** Blood Cholesterol [6], Blood Urea [6], Serum Uric Acid [6], Blood Creatinine [6] and Serum protein [6] were estimated by standard procedures.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

The body weight of the rats before and after administrations were noted that there is slightly increased the body weight. But there are no changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also no sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also not there. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. The acute toxicity study in rats showed that at 2000 mg/kg dose, the plant is safe for consumption and for medicinal uses (Table 1).

Chronic toxicity study

The chronic oral administration of ethanolic extract of *Acacia visco L.* used no noticeable change in the general behavior of the rats and, compared to the control group (saline and vehicle), no significant changes in body weight, food intake and utilization of food in the EEAV treated rats. Both the control and treated rats appeared uniformly healthy at the end and throughout the six weeks period of study.

In the chronic toxicity study, the haematological parameters, hemoglobin concentration, clotting time, neutrophils, easinophils, lymphocytes, monocytes, red and white blood cells in the treated rats did not differ significantly ($P > 0.01$) from that of the control group

(Table 2) and all the values remained within normal limits throughout the experimental period. As shown in Table 3 & 4, no significant treatment-related changes in the levels of hepatic and renal parameters such as SGOT, SGPT, cholesterol, creatinine, urea, uric acid, protein and glucose, and serum ALP activities were observed at the termination of the study. In this study, the ethanol extract of Acacia visco L. was found to be non-toxic in rats when administered orally in doses up to 2000 mg mg/kg, p.o. The onset of toxicity and signs of toxicity also not there. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. Based on this animal study, may be described as being practically non-toxic.

Table 1: Acute toxicity study of ethanol extract of *Acacia visco* L. (EEAV) in rats

S.No	Groups	Dose/kg b.w	Weight of animals		Signs of Toxicity	Onset of Toxicity	Duration of study
			Before Test	After Test			
1	EEAV	2000 mg	169 g	168 g	No signs of Toxicity	Nil	14days
2	EEAV	2000 mg	175 g	176 g	No signs of Toxicity	Nil	14days
3	EEAV	2000 mg	183g	183 g	No signs of Toxicity	Nil	14days
4	EEAV	2000 mg	185 g	186 g	No signs of Toxicity	Nil	14days
5	EEAV	2000 mg	211 g	210 g	No signs of Toxicity	Nil	14days
6	EEAV	2000 mg	202 g	203 g	No signs of Toxicity	Nil	14days

Table 2. .Effect of ethanol extract of Acacia visco L. (EEAV) on heamotological profiles in rats

Design of treatment	Group I Saline (0.9 % W/V)	Group II Vehicle (1%SCMC)	Group III EEAV	Group IV EEAV	Group V EEAV
Dose mg/kg	5 ml/kg,p.o	5 ml/kg,p.o	100mg/kg,p.o	200mg/kg,p.o	400mg/kg,p.o
Neutrophil (%)	21.4± 0.63	26.7 ± 0.42	35.74±1.13[a]	38.90 ± 0.74[a]	40.56± 0.80[a]
Eosinophil (%)	2.6± 0.34	1.03± 0.69	3.51± 0.56[a]	0.93 ± 0.85[a]	1.02± 0.47[a]
Lymphocyte (%)	71.58± 0.56	71.4± 1.05	68.69± 3.04[a]	60.54 ± 2.39[a]	54.7±3.16[a]
Monocyte (%)	4.23±1.07	3.01± 0.39	5.8 ± 0.95[a]	3.79 ± 1.14[a]	2.38 ± 1.8[a]
Clotting time (seconds)	79.19± 4.28	80.15±2.24	93.06±3.78[a]	98.1 ± 2.37[a]	101.4±2.43[a]
Haemoglobin (gm%)	14.67± 0.19	15.39 ±1.56	13.24± 1.02[a]	13.82± 0.49[a]	13.5± 1.68[a]
RBC cells (cu.mm)×10^9(%)	9.46± 0.45	8.52 ±0.93	9.36± 1.27[a]	7.9± 0.82[a]	7.35 ± 0.79[a]
WBC cells (cu.mm)×10^9 (%)	7.38± 0.72	9.64±1.12	8.03 ± 0.39[a]	10.21±0.58[a]	11.9± 0.24[a]

a-Group I & II Vs group III, IV &V. P < 0.01 when compared to control group
Each value represents the mean ± S.E.M six rats in each group

Table 3. Effect of ethanol extract of Acacia visco L. (EEAV) on hepatic parameters in rats

Groups	Design of treatment	Dose Mg/kg	Glucose Mg/dl	Bilirubin Mg/dl	SGOT 1 Unit/L	SGPT 1 Unit/L	ALP 1 Unit/L
I	Saline(0.9 % W/V)	5 ml /kg,p.o	88± 4.12	0.5± 0.004	52.7 ±0.9	32.3 ±0.8	9.1 ±0.93
II	Vehicle (1% SCMC)	5ml/kg,p.o	97± 5.24	0.7 ±0.002	58.84 ±0.6	35.91 ±2.4	10.03 ±0.92
III	EEAV	100mg/kg,p.o	99±4.8[a]	0.9± 0.003[a]	54.56 ±0.8[a]	35.78 ±0.7[a]	11.67 ±0.41[a]
IV	EEAV	200mg/kg,p.o	102±5.56[a]	0.6 ± 0.011[a]	56.01 ±0.5[a]	37.0 ±0.6[a]	12.82 ±0.78[a]
V	EEAV	400mg/kg,p.o	104±4.93[a]	0.8 ±0.009[a]	59.92 ± 0.7[a]	38.1 ±0.8[a]	13.50 ±1.0[a]

Each value represents the mean ± S.E.M six rats in each group

Table 4. Effect of ethanol extract of Acacia visco L. (EEAV) on renal parameters in rats

Groups	Design of treatment	Dose mg/kg	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl	Protein gm/dl
I	Saline(0.9 %)W/V	5 ml/kg,p.o	22 ± 0.93	5.0 ± 0.8	0.13 ± 0.002	7.4±0.89
II	Vehicle (1%SCMC)	5 ml/kg,p.o	23 ± 0.72	6.8 ± 0.9	2.4± 0.004	8.1±0.93
III	EEAV	100mg/kg,p.o	24 ± 0.85[a]	4.9 ± 0.7[a]	3.2±0.003[a]	7.8± 1.02[a]
IV	EEAV	200mg/kg,p.o	27 ± 0.99[a]	5.6±0.11[a]	2.8±0.005[a]	8.2 ± 1.06[a]
V	EEAV	400mg/kg,p.o	29 ± 1.02[a]	4.2±0.24[a]	3.6±0.007[a]	7.3 ± 0.78[a]

a-Group I & II Vs group III, IV &V. P < 0.01 when compared to control group
Each value represents the mean ± S.E.M six rats in each group

In the six weeks chronic toxicity study, the EEAV at the doses of 100, 200 & 400mg/kg did not appear to affect the bodyweight or the behavior of the rats and caused no significant changes in their food intake and utilization of food indicating normal metabolism in the animals and suggesting that, at the oral doses administered EEAV did not retard the growth of rats. After six weeks treatment, there were also no treatment related changes in the haematological parameters (i.e. hemoglobin concentration, clotting time, neutrophils, easinophils, lymphocytes, monocytes, red and white blood cells) between control and treated groups indicating that the EEAV was not toxic to the circulating red cells, nor interfered with their production. Hematopoiesis and leucopoiesis were also not affected even though the haematopoietic system is one of the most sensitive targets for toxic compounds [13] and an important index of physiological and pathological status in man and animals [14]**.**

CONCLUSION

The present investigation demonstrates that at doses consumed in the traditional medicine, the ethanol extract of Acacia visco L. may be considered as relatively safe, as it did not cause either any lethality or changes of in the general behavior in both the acute and chronic toxicity studies in rats. Studies of this type are needed before a phytotherapeutic agent can be generally recommended for use.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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