Acute and Chronic Toxicity Studies of Ethanolic Extract of *Alocassia Macrorhiza* (L.) Leaves in Experimental Animals.

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Summary

The present investigation was carried out to evaluate the safety of an ethanol extract of *Alocassia macrorhiza* (L.) (EEAM) leaves by determining its potential toxicity after acute and chronic administration in rats. Study on acute toxicity of extract found to be safe at the doses 2000mg/kg body weight orally as per OECD guidelines No.423. General behavior adverse effects and mortality were determined for up to 14 days. In the chronic toxicity study, the EEAM was administered orally at doses of 100, 200 and 400 mg/kg once in a week for 6 weeks to rats. Biochemical and hematological parameters were determined after 6 weeks. In the acute study in rats, there was no toxicity/ death was observed at the dose of 2000mg/kg b.w. The onset of toxicity and signs of toxicity also not there. In the chronic toxicity study, no significant treatment-related changes in the levels of haematological, 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. It suggests that the ethanol extract of *Alocassia macrorhiza* (L.) does not appear to have significant toxicity. In view of the dose of *Alocassia macrorhiza* consumed in traditional medicine, there is a wide margin of safety for the therapeutic use of the ethanol extract of *Alocassia macrorhiza* (L.) leaves.

Key words: *Alocassia macrorhiza* (L.), Traditional Medicine, Acute and Chronic Toxicity, Heamatological Parameters, Biochemical Parameters.

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Introduction

***Alocassia macrorhiza* (Linn.)** (Family: Araceae) is probably native to Indo-malesia but widely distributed by aboriginal peoples throughout South-East Asia into the tropical Pacific. According to literature review its constituents**,** oxalic acid, calcium oxalate, flavonoids, cyanogenic glycosides, alocasin, cholesterol, beta-sitosterol, stigmatosterol, camposterol, fucosterol, amino acids, citric acid, malic acid, ascorbic acid, succinic acid, glucose, fructose and sucrose. Arabino-galactan proteins and betalectins [1,2]. This plant traditionally using, In Fiji, the sap of the stem is used to treat ear ache or boils in the ear. The wood is used to treat stomachache and diarrhoea. In New Guinea, headaches are treated with the sap and the leaves. Sexual disorders are treated by eating the leaves cooked in coconut milk [1,3].In spite of the use of *Alocassia macrorhiza* in traditional medicine and its potential for toxicity, systematic evaluation of its toxic effects is lacking. Therefore, the aim of the present study was to investigate the acute and chronic toxic effects of an ethanol extract of *Alocassia macrorhiza* in rodents.

Materials and Methods

Plant collection

The Plant material of *Alocassia macrorhiza* (L.) leaves used for investigation and it was collected from Tirunelveli District, in the Month of December 2008. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen (CHE-SA-AM-012) of the plant was deposited at the college for further reference.

Preparation of extracts

Alocassia macrorhiza (L.) leaves were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (80gm) 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 *Alocassia macrorhiza* (L.) was found to be 15.5 % w/w.

Animals used

Male Albino wistar rats (150-230g) of either sex were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati. The 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 (Ref No. IAEC / XIII / 05 / SVCP / 2008 - 2009).

Toxicological evaluation of *Alocassia macrorhiza* (L.) extract in rats

Acute toxicity study of *Alocassia macrorhiza* (L.) extract in rats

The procedure was followed by using OECD 423 (Acute Toxic Class Method). [4] 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 EEAM 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 for 14 days. 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.

Study of Chronic Toxicity of *Alocassia macrorhiza* (L.) extract in rats

Design of Treatment

Animals were divided into 5 groups of six rats each.

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

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

Group III-V- Ethanolic extract of Alocassia *macrorhiza* (L.) leaves 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.

Collection of blood and serum samples

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.

Methods for estimation of haematological parameters

Estimation of Hemoglobin [6], RBC count [6], WBC count [6], different leucocytic count [5], Elongation time [5] and ESR [7] were determined according to the standard procedures.

Determination of serum biochemical parameters

Blood Glucose,[8] Serum Bilirubin [9], Serum Gluconate – Oxaloacetate Transaminase (SGOT) [9], Serum Glutamate – Pyruvate Transaminase (SGPT) [9], Serum Alkaline Phosphatase (ALP) [9], Blood Cholesterol [8], Blood Urea [8], Serum Uric Acid [8], Blood Creatinine [8] and Serum protein[8] 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 analysis 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)**

Table 1. Acute toxicity study of ethanol extract of *Alocassia macrorhiza* (L.)(EEAM) in rats

S.No	Groups	Dose/kg b.w, p.o	Weight of animals		Signs of Toxicity	Onset of Toxicity	Duration of study
			Before Test	After Test			
1	EEAM	2000 mg	160 g	160 g	No signs of Toxicity	Nil	14days
2	EEAM	2000 mg	180 g	182 g	No signs of Toxicity	Nil	14days
3	EEAM	2000 mg	162g	164 g	No signs of Toxicity	Nil	14days
4	EEAM	2000 mg	180 g	186 g	No signs of Toxicity	Nil	14days
5	EEAM	2000 mg	210 g	207 g	No signs of Toxicity	Nil	14days
6	EEAM	2000 mg	202 g	206 g	No signs of Toxicity	Nil	14days

Chronic toxicity study

The chronic oral administration of ethanolic extract of Alocassia *macrorhiza* (L.) leaves caused no noticeable change in the general behaviour 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 EEAM treated rats. Both the control and treated rats appeared uniformly healthy at the end and throughout the six weeks period of study.

Effect of ethanolic extract of Alocassia macrorhiza (L.) leaves on the haematological and biochemical parameters of rats

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.

Discussion and conclusion

A Word Health Organization survey indicated that about 70–80% of the world's populations rely on non-conventional medicine, mainly of herbal source, in their primary healthcare**.**[10,11] Although medicinal plants may produce several biological activities in humans, generally very little is known about their toxicity and the same applies for Alocassia macrorhiza (L.). Because safety should be the overriding criterion in the selection of medicinal plants for use in healthcare systems [12]. To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dose in humans [13]. One should, in addition to the use of historical documentation on Alocassia macrorhiza, also have formal toxicological evaluations of this plant to optimize its safe use as a medicine. The ethanol extract of Alocassia macrorhiza (L.) used in the present study offers several advantages as a form of the Alocassia macrorhiza (L.) medicine.[14] But before such evaluation can be fully justified in humans, the preclinical evaluation of the safety of the Alocassia macrorhiza (L.) is required.

Table 2. .Effect of ethanol extract of *Alocassia macrorhiza* (l.) (EEAM) on heamotological profiles in rats

Design of treatment	Group I Saline(0.9 % W/V)	Group II Vehicle (1%SCMC)	Group III EEAM	Group IV EEAM	Group V EEAM
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 (%)	22.1± 0.48	25.2 ± 0.47	32.6 ± 0.43[a]	37.8 ± 0.51[a]	39.2 ± 0.47[a]
Eosinophil (%)	1.0 ± 0.02	0.9 ± 0.04	1.0 ± 0.04[a]	0.8 ± 0.04[a]	0.8 ± 0.02[a]
Lymphocyte (%)	70.9 ± 0.72	70.3 ± 0.68	67.4 ± 1.18[a]	59.4 ± 1.12[a]	53.6 ± 1.47[a]
Monocyte (%)	3.1 ± 0.57	2.9 ± 0.49	2.7 ± 0.21[a]	2.6 ± 0.44[a]	1.9 ± 0.57[a]
Clotting time (seconds)	77.1 ± 1.77	79.2 ± 1.77	92.1 ± 1.81[a]	97.8 ± 1.69[a]	99.2 ± 1.71[a]
Haemoglobin (gm%)	13.4 ± 0.15	13.2 ± 0.11	12.7 ± 0.12[a]	12.6 ± 0.11[a]	12.3 ± 0.14[a]
RBC cells (cu.mm)×10^9(%)	7.4 ± 0.10	7.3 ± 0.11	7.2 ± 0.9[a]	6.8 ± 0.12[a]	6.7 ± 0.11[a]
WBC cells (cu.mm)×10^9 (%)	6.8 ± 0.17	7.7 ± 0.14	7.9 ± 0.22[a]	8.4 ± 0.21[a]	$^{9.2}$ ± 0.17[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 *Alocassia macrorhiza* (l.) (EEAM) on hepatic parameters in rats

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

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	Cholestrol mg/100ml
I	Saline(0.9 % W/V)	5 ml /kg,p.o	88 ± 3.5	0.4 ± 0.001	50.8 ±0.7	32.1 ±0.7	8.3 ±0.37	60.7 ±1.8
II	Vehicle (1% SCMC)	5ml/kg,p.o	97 ± 3.2	0.6 ±0.001	57.2 ±0.4	34.1 ±1.5	8.7 ±0.33	66.6 ±1.4
III	EEAM	100mg/kg,p.o	99 ± 3.7[a]	0.5 ± 0.001[a]	53.1 ±0.6[a]	35.2 ±0.6[a]	10.2 ±0.37[a]	54.2 ±1.7[a]
IV	EEAM	200mg/kg,p.o	102 ± 3.4[a]	0.6 ± 0.001[a]	55.2 ±0.4[a]	37.6 ±0.4[a]	11.1 ±0.32[a]	59.1 ±1.6[a]
V	EEAM	400mg/kg,p.o	104 ± 3.1[a]	0.7 ±0.001[a]	57 ± 0.6[a]	38.0 ±0.6[a]	12.1 ±0.81[a]	69.7 ±1.5[a]

Table 4. Effect of ethanol extract of *Alocassia macrorhiza* (l.) (EEAM) 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	20 ± 0.55	4.1 ± 0.7	0.9 ± 0.001	6.7 ±0.10.
II	Vehicle (1%SCMC)	5 ml/kg,p.o	22 ± 0.44	4.3 ± 0.8	1.2 ± 0.001	6.9 ±0.12
III	EEAM	100mg/kg,p.o	24 ± 0.57[a]	3.9 ± 0.6[a]	1.1 ±0.001[a]	6.9 ± 0.14[a]
IV	EEAM	200mg/kg,p.o	27 ± 0.54[a]	3.8 ±0.7[a]	1.2 ±0.001[a]	7.1 ± 0.37[a]
V	EEAM	400mg/kg,p.o	29 ± 0.56[a]	3.7 ±0.6[a]	1.4 ±0.001[a]	7.3 ± 0.31[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 this study, the ethanol extract of Alocassia macrorhiza (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.

In the six weeks chronic toxicity study, the EEAM at the doses of 100, 200 & 400mg/kg did not appear to affect the bodyweight or the behaviour 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 EEAM 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 EEAM 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 [15] and an important index of physiological and pathological status in man and animals [16].

In addition, most of the hepatological and renal parameters (i.e. Glucose, creatinine, Bilirubin, SGOT, SGPT, ALT, urea, uric acid, protein and cholesterol,) were also unchanged by the doses of EEAM 100, 200 & 400mg/kg. The lack of significant alterations in the levels of ALP, creatinine, Bilirubin, SGOT, SGPT and cholesterol, good indicators of liver and kidney functions, respectively[17]. The transaminases (SGOT and SGPT) are well known enzymes used as biomarkers predicting possible toxicity [18]. Generally, damage to the parenchymal liver cells will result in elevations of both these transaminases [19]. The transaminases were not significantly increased at the doses of EEAM 100, 200 & 400mg/kg. It suggests that chronic ingestion of EEAM did not alter the hepatocytes and kidneys of the rats, and, furthermore the normal metabolism of the animals. The relevance of this result may be associated with the biological value of the plant Alocassia macrorhiza (L.).

In conclusion, the present investigation demonstrates that at doses consumed in the traditional medicine, the ethanol extract of Alocassia macrorhiza (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.

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