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ACUTE AND SUBACUTE TOXICITY STUDY OF THE ETHANOLIC EXTRACT FROM CALOTROPIS GIGANTEA R.BR. IN RODENTS

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ABSTRACT

Acute and subacute toxicity of the ethanolic extract from *Calotropis gigantea* R.Br. flower was investigated. In acute toxicity study oral dose of 2000 mg/kg of the ethanolic extract did not produce mortality or changes in the general behavior and gross appearance of internal organs of mice and rats. In subacute toxicity study, ethanolic extract was evaluated at 250, 500 or 1000 mg/kg/day, orally for 30 days in rats. The behavioral response profile of the treated mice and rat was also evaluated along with other parameters such as, absolute and relative body weight along with relative weight of various organs. Biochemical and hematological parameters were analyzed in order to study the time-dependent effect and correlation of the extract. All treated animals did not show any signs of toxicity during the experimental period. There were no significant differences in the body and organ weights between the control and the treated group of mice and rats. Hematological (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LYM and NEUT) and biochemical parameters (AST, ALT, ALP, ALBN, TB, TP, TG, BUN, creatinine and glucose) revealed either no or less alteration in the treated group. Hence, the *C. gigantea* was found to be safe in single dose acute and repetitive dose subacute toxicity studies.

KEYWORDS

Calotropis gigantea, Acute toxicity, Subacute toxicity, Mice and Rats

INTRODUCTION

The *Calotropis gigantea* is a perennial shrub belonging to the Asclepiadaceae family found chiefly in wastelands throughout India, in comparatively drier and warmer areas, upto an altitude of 1050 meters [1]. It is called as "Ruvi" in Marathi and "Madar" in Hindi. Flowers are regular, bisexual, arranged in simple or rarely compound cymose corymbs [2]. It has been reported traditionally for antifertility, alexipharmic, antihelmintic, purgative and abortifacient activities. The plant has enormous medicinal properties to cures leprosy, leucoderma, ulcers, tumours, piles, diseases of the spleen, liver and abdomen. In folklore medicines *C. gigantea* roots are reported for their analgesic, anticonvulsant, anxiolytic and sedative properties [2]. Previous reports suggest the central nervous system stimulant [1] and pregnancy interceptive potentials of root bark [3]. The juice is anthelmintic, laxative; cures piles and "kapha". The milk is bitter, oleaginous, purgative;

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cures leucoderma, tumours, ascites and diseases of the abdomen.

The flowers are astringent, bitter and claimed to cure asthma, eczema, leprosy, secondary syphilis, gonorrhea, ascites, helminthiasis, diarrhoea and jaundice in Ayurveda, Siddha and Unani literature [4, 5]. The flowers of *C. gigantea* are reported to have hepatoprotective and analgesic activity in mice [6, 7]. Other experiments demonstrated the anti-inflammatory [8, 9] and anti-ulcer activity [9].

The major constituents responcible for medicinal properties of *Calotropis gigantea* are flavonol glycoside, akundarol, uscharidin, calotropin, frugoside, calotroposides A to G. Whereas, other constituents are α-amyrin, β-amyrin, taraxasterol, β-sitosterol, α-amyrin methylbutazone, β-amyrin methylbutazone, α-amyrin acetate, β-amyrin acetate, taraxasteryl acetate, lupeol acetate B, gigantursenyl acetate A, gigantursenyl acetate B [10, 11].

Since different parts of the plant have immense potential to cure various diseases and disorders, it is used in various polyherbal preparations [7, 12]. However, the complete toxicological profile is still not generated. So the aim of this study was to evaluate the acute and subacute toxicity of the ethanolic extract of *C. gigantea* (CGFE) in mice and rats. In the acute and subacute toxicity study the effect on biochemical, hematological and histopathological parameters were investigated.

MATERIALS AND METHODS

(i) *Plant Material*

The flowers of *Calotropis gigantea* was procured from Regional Research Institute (Ay.) Govt. Central Pharmacy Annex, Bangalore-560011. The authentication of plant was done by Dr. Siddamallayya and it is stored in herbarium with reference no. *RRI/ BNG/ SMP/, 2007-08/ 964* (RRCBI Acc. No. 3276).

(ii) *Extraction*

The shade dried flowers of about 2 kg were subjected for size reduction to coarse powder. The powder was defatted with petroleum ether (60–80^{0}C) and then extracted with 5 L of 90% ethanol using Soxhlet apparatus. The ethanolic extract was concentrated under vacuum to get the residue. After recovery of ethanolic extracts total 34.9 % w/w yield was obtained. The extract was stored in air tight container at -20 ^{0}C in deep freezer till further use.

(iii) *Animals*

Albino Swiss mice (20-35 g) and albino Wistar rats (250-300 g) of either sex were housed in polyethylene-walled cages. The animals were kept on a 12 h light: 12 h dark regime (lights on from 7:00 h to 19:00 h) at 23 °C prior to the experiments. The animals had free access to water and standard diet. Mice and rats were deprived of food but not water 3 and 12 h prior to administration of the test substances respectively.

(iv) *Acute toxicity*

Healthy nulliparous and non-pregnant adult albino Swiss mice (20- 35g) and adult albino Wistar rats (250-300) were subjected to acute toxicity studies as per guidelines (425) suggested by the OECD 2001 [13]. Two groups of three albino Swiss mice and albino Wistar rats were given single oral doses of 2000 mg/kg b.w. of the CGFE. The control group received distilled water at the same volume.

Observations were made systematically and recorded continuously for initial one h and occasionally for subsequent four h followed by observation after 24 h of administration of test substance. The visual observations included changes in the skin and fur, eyes and mucous membranes and also respiratory,

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circulatory, autonomic and central nervous system as well as somatomotor activity and behavioral pattern. The number of survivors was noted after 24 h and these were then maintained for a further 14 days with a once daily observation. At the conclusion of experiment, all surviving animals were fasted and anesthetized with anesthetic ether and blood samples were collected retro-orbitally into heparinized tubes. The heparinized blood was used for hematological study included WBC and differential leukocyte count, platelet, hematocrit and hemoglobin estimation. Also the gross morphology of internal organs such as lungs, livers, kidneys and spleen were studied.

(v) ***Subacute toxicity***

As per the acute toxicity study, CGFE was found to be safe at 2000mg/kg of b.w. To study the effect of prolong exposure of extracts at therapeutic doses; the sub-acute oral toxicity study was performed according to the OECD test guidelines with slight modifications [14].

Four groups of 10 rats received the ethanolic CGFE at the doses of 250, 500 or 1000 mg/kg/day or distilled water (control) for 30 consecutive days. During the period of administration, the animals were weighed and observed daily to detect signs of toxicity. Daily visual observations were made and recorded systematically similar to those performed as in the case acute toxicity study. At the end of experimental period, all surviving animals were fasted before anesthetization with anesthetic ether. Blood samples were collected retro-orbitally into heparinized and dry non-heparinized centrifuge tubes. The heparinized blood was used for hematological study included WBC and differential leukocyte count, platelet, hematocrit and hemoglobin estimation. The serum was separated from the non-heparinized blood and was assayed for glucose, serum urea (BUN), creatinine (Cr), triglycerides (TG), total protein (TP), albumin (ALBN), total globulin (Glob), total billirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). After blood collection animals were sacrificed for histopathological studies. The organs such as brain, heart, kidneys, livers, lungs and spleen were removed, blotted free of blood and weighed immediately on an electronic balance and used for subsequent analysis.

(vi) ***Statistical analysis***

The results were expressed as mean ± S.E.M. Statistical significance was determined by one-way ANOVA test. The data obtained from acute toxicity study were analyzed using Dunnett Multiple Comparison Test. *P*-values less than 0.05 were considered significant.

RESULTS

1. *Acute toxicity study*

Table 1.

***Effect of C. gigantea on change in body weight in single dose (2000 mg/kg) 14 days study in mice* and rats**

Parameter (Body weights)	Control (Mice)	CGFE (Mice)	Control (Rats)	CGFE (Rats)
Before	30.04 ± 1.92	25.78 ± 1.06	277.79 ± 7.22	273.88 ± 5.33

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After	29.99 ± 1.39	26.48 ± 1.01	283.93 ± 8.27	280.63 ± 4.83

Values are expressed as mean ± S.E.M., (n= 3), ANOVA followed by Dunnett's Multiple Comparison Test
No significant difference was observed between control and test groups.

Table 2.
Effect of C. gigantea extracts on relative organ weight in single dose (2000 mg/kg) 14 days study in mice and rats

Parameter (Relative organ weight 2g/kg b.w.)	**Control (Mice)**	***CGFE* (Mice)**	**Control (Rats)**	***CGFE* (Rats)**
Kidney	1.35 ± 0.01	1.37 ± 0.01	1.60 ± 0.21	1.34 ± 0.18
Liver	2.85 ± 0.11	2.52 ± 0.08	9.63 ± 0.48	9.06 ± 0.37
Lung	0.53 ± 0.01	0.54 ± 0.03	1.41 ± 0.19	1.14 ± 0.14
Spleen	0.39 ± 0.01	0.33 ± 0.01	0.69 ± 0.04	0.61 ± 0.04

Values are expressed as mean ± S.E.M., (n= 3), ANOVA followed by Dunnett's Multiple Comparison Test
No significant difference was observed between control and test groups.

Table 3.
Effect of C. gigantea on hematological parameters in single dose (2000 mg/kg) 14 days study in mice

Hematological parameter	***Control***	***CGFE***
WBC (x 10^3 mm $^{-3}$) [a]	7.88 ± 0.31	7.54 ± 0.14
RBC (x 10^6 mm $^{-3}$) [b]	7.44 ± 0.15	7.31 ± 0.13
HGB (g/dl) [c]	11.42 ± 0.16	11.45 ± 0.14
HCT (%) [d]	38.75 ± 1.12	40.68 ± 1.20
MCV (fl) [e]	55.89 ± 1.85	56.25 ± 1.16
MCH (pg) [f]	16.24 ± 0.92	15.88 ± 1.06
MCHC (gm/dl) [g]	29.51 ± 1.15	27.94 ± 0.91
PLT (x 10^3 mm $^{-3}$) [h]	837.83 ± 54.44	885.33 ± 40.46
LYM (%) [i]	83.48 ± 3.57	72.21 ± 4.67
NEUT [j]	18.16 ± 1.95	16.83 ± 2.38

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Values are expressed as mean ± S.E.M., (n=3), ANOVA followed by Dunnett's Multiple Comparison Test
No significant difference was observed between control and test groups.
[a] White blood cell ($\times 10^3$ mm^{-3}), [b] Red blood cell ($\times 10^6$ mm^{-3}), [c] Hemoglobin concentration (g/dl), [d] Hematocrit value (%) ,[e] Mean corpuscular volume (fl),[f] Mean corpuscular hemoglobin (pg),[g] Mean corpuscular hemoglobin concentration (g/dl), [h] Platelets ($\times 10^3$ mm^{-3}), [i] Lymphocyte (%), [j] Neutrophils.

Table 4.

Effect C. gigantea on hematological parameters in single dose (2000 mg/kg) 14 days study in rats

Hematological parameter	*Control*	*CGFE*
WBC (x 10^3 mm^{-3}) [a]	9.96 ± 1.04	9.56 ± 0.51
RBC (x 10^6 mm^{-3}) [b]	8.44 ± 0.49	8.70 ± 0.45
HGB (g/dl) [c]	15.04 ± 0.11	14.92 ± 1.89
HCT (%) [d]	38.74 ± 1.86	36.89 ± 1.93
MCV (fl) [e]	52.89 ± 1.94	50.23 ± 2.11
MCH (pg) [f]	18.95 ± 1.21	19.40 ± 1.64
MCHC (gm/dl) [g]	30.61 ± 2.82	29.63 ± 2.40
PLT (x 10^3 mm^{-3}) [h]	1011.41± 64.74	926.10 ± 37.86
LYM (%) [i]	69.67 ± 3.48	66.94 ± 2.96
NEUT [j]	21.92 ± 1.77	19.85 ± 2.16

Values are expressed as mean ± S.E.M., (n = 3), ANOVA followed by Dunnett's Multiple Comparison Test
No significant difference was observed between control and test groups.
Where,
[a] White blood cell ($\times 10^3$ mm^{-3}), [b] Red blood cell ($\times 10^6$ mm^{-3}), [c] Hemoglobin concentration (g/dl), [d] Hematocrit value (%) , [e] Mean corpuscular volume (fl),[f] Mean corpuscular Hemoglobin (pg), [g] Mean corpuscular Hemoglobin concentration (g/dl), [h] Platelets ($\times 10^3$ mm^{-3}), [i] Lymphocyte (%), [j] Neutrophils

In the acute toxicity test dose of 2000 mg/kg of the ethanolic extract of *C. gigantea* did not cause mortality in mice and rats during 14-days observation. The mice and rats did not show any signs of toxicity or change in general behavior or other physiological activities. The slight change in body weight of the mice and rats treated with the CGFE is not significant as compared with that of the control group (Table 1). There were no differences in the weight of the internal organs of mice and rats in the control and the treated groups (Table 2). The hematological examination of the blood from mice (Table 3) and rats (Table 4) revealed that there were no signs of abnormalities.

2. *Subacute toxicity study*

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Table 5.

Effect of C. gigantea extracts on relative organ weight in daily dose 30 days study in rats

Parameter (Relative organ weight g/100 b.w.)	Control	*CGFE* 250	*CGFE* 500	*CGFE* 1000
Brain	1.65 ± 0.06	1.59 ± 0.08	1.66 ± 0.12	1.66 ± 0.02
Heart	0.90 ± 0.03	0.93 ± 0.04	0.98 ± 0.03	0.94 ± 0.04
Kidney	2.30 ± 0.13	2.36 ± 0.14	2.23 ± 0.11	2.32 ± 0.22
Liver	9.04 ± 0.06	9.15 ± 0.10	8.88 ± 0.11	9.02 ± 0.04
Lung	2.87 ± 0.08	2.75 ± 0.14	2.92 ± 0.16	2.88 ± 0.24
Spleen	0.69 ± 0.03	0.66 ± 0.06	0.68 ± 0.07	0.69 ± 0. 02

Data expressed as mean ± S.E.M., n= 10, ANOVA followed by Dunnett's Multiple Comparison Test

No statistical difference between control and test groups ($p > 0.05$)

Table 6

Effect of C. gigantea extracts on hematological parameters in daily dose 30 days study in rats

Hematological Parameter	*Control*	*CGFE 250*	*CGFE 500*	*CGFE 1000*
WBC (x 10^3 mm^{-3}) [a]	10.01±1.1	9.32 ± 0.7	10.27±0.4	10.22± 1.2
RBC (x 10^6 mm^{-3}) [b]	8.42 ± 0.7	8.72 ± 0.7	8.97 ± 0.7	9.01 ± 0.8
HGB (g/dl) [c]	15.12±0.8	14.59± 1.1	13.98± 1.3	14.8 ± 0.6
HCT (%) [d]	42.8 ±1.4	44.5 ± 0.9	43.5 ± 0.61	42.6 ± 1.6
MCV (fl) [e]	51.01±0.8	50.8 ± 0.7	51.01± 0.9	50.01 ± 0.8
MCH (pg) [f]	18.31±0.6	18.11± 0.5	18.96± 0.8	18.24 ± 0.6
MCHC (gm/dl) [g]	34.47±1.0	32.59± 1.3	33.80± 1.2	36.44 ± 2.5
PLT (x 10^3 mm^{-3}) [h]	922.5±37.3	848.9±34.0	809.2±63.7	928.6 ±33.2

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LYM (%) [i]	69.96± 0.9	69.17±1.05	74.17± 5.1	66.84 ± 5.1
NEUT [j]	21.69±1.0	22.49± 1.10	22.02± 1.3	20.98 ±1.2

Values are expressed in mean ± S.E.M., (n = 10), ANOVA followed by Dunnett's Multiple Comparison Test
No significant difference was observed between control and test groups.
Where,
[a] White blood cell ($\times 10^3$ mm^{-3}), [b] Red blood cell ($\times 10^6$ mm^{-3}), [c] Hemoglobin concentration (g/dl), [d] Hematocrit value (%), [e] Mean corpuscular volume (fl), [f] Mean corpuscular Hemoglobin (pg), [g] Mean corpuscular Hemoglobin concentration (g/dl), [h] Platelets ($\times 10^3$ mm^{-3}), [i] Lymphocyte (%), [j] Neutrophils.

Table 7.
Effect of the C. gigantea on serum biochemical parameters in rats treated for 30 days

Parameter	**Control**	***CGFE***		
		250mg/kg	**500mg/kg**	**1000 mg/kg**
AST (IU/L)	86.5±5.8	79.8±5.3	84.0±8.2	97.4± 6.2
ALT(IU/L)	176.1 ±4.6	179.2 ± 4.0	182.2 ± 4.6	186.6 ± 3.2
ALP(IU/L)	162.8±9.1	154.8±8.9	161.5±6.8	168.2 ± 8.6
ALBN (gm/dL)	3.15±0.1	3.01±0.1	2.93±0.2	3.02 ± 0.1
TB (mg/dL)	2.08 ± 0.1	2.05±0.2	2.03±0.2	2.06 ± 0.2
TP (gm/dL)	6.33 ± 0.3	6.71±0.4	6.91±0.5	6.51 ± 0.2
TG (mg/dL)	52.3±2.2	58.2±5.7	59.8±3.5	54.2 ± 1.6
BUN (mg/dL)	43.8±1.6	43.5±3.0	46.3±2.1	40.6 ± 3.1
Creatinine(mg/dL)	0.47±0.0	0.49±0.0	0.43±0.0	0.42 ± 0.0
Glucose (mg/dL)	66.2±2.8	64.9±4.0	67.5±3.3	64.4 ± 3.6

Data expressed as ± S.E.M., (n= 10), ANOVA followed by Dunnett's Multiple Comparison Test
No statistical difference between control and test groups ($p > 0.05$).
Where,
AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, ALBN: Albumin, TB: Total bilirubin, TP: Total protein,
TG: Triglycerides, BUN: blood urea nitrogen, Creatinine, glucose.

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In the subacute toxicity study, the CGFE at doses of 250, 500 or 1000 mg/kg, given orally for 30 days, did not produce any mortality in rats. No signs of toxicity were observed during the experimental period. The body weight changes of the CGFE treated animals were not significant with respect to the control animals. As shown in Table 5, the organ weights of the CGFE-treated rats did not differ from those of the control group.

The hematological values of treated rats were not significantly different from those of the control group (Table 6). However, platelet count in the animal treated group was slightly lower than that of the control group.

Blood chemistry values of rats are shown in Tables 7. The values of biochemical markers like AST, ALT, ALP, TP, ALBN, TB, BUN, creatinine, glucose and TG of CGFE treated group were slightly changed when compared with those of the control group.

DISCUSSION

In present study, the administrations of *Calotropis gigantea* flowers extract up to dose of 2000 mg/kg b.w. p.o. did not produce any mortality or significant alteration in the behavioral pattern of mice and rats as compared to the control group. The change in weights of mice and rats in treated group were not significant as compared with control animals. According to the OECD guideline for testing of chemicals, the results of acute toxicity test in this study indicate that the flowers of *Calotropis gigantea* are fairly non-toxic. The present work indicated that in subacute study the CGFE at 250, 500 or 1000 mg/kg/day for 30 consecutive days did not cause any mortality in the rats, although it caused slight change in body weight of rats (data not shown). Also, the histopathology of all organs was found to be normal and the weights of the internal organs were not found to be significantly different from the control groups. Some of the hematological and the differential white blood cell values of the CGFE treated animals differed slightly from those of the control group, but some values were in the ranges of the control. These effects may therefore be due to variations in the animals.

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