



PRELIMINARY PHYTOCHEMICAL STUDY AND SAFETY PROFLIE OF *CLERODENDRUM SERRATUM*

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ABSTRACT

The present study was aimed to evaluate phytochemical constituents and the safety of aqueous and Methanolic extract of aerial parts of *Clerodendrum serratum* (AECS & MECS) by determining their potential toxicity after acute and 28-days repeated dose administration in Albino rats. The phytochemical analysis was done by standard laboratory grade reagents. Acute and 28-day repeated dose oral toxicity studies were performed by following OECD test guide lines 423 and 407 respectively. The present study reveals the presence of complex phytochemical constituents like flavonoids, carbohydrate, tannins, terpenoids and steroids. In acute toxicity study no treatment related death or toxic signs were observed with AECS and MECS administration. In repeated dose study no significant difference in haematological parameter were observed between control and AECS and MECS groups. Controversially there was a slight an increase in the bodyweight, serum cholesterol and protein and slightly reduce the ALP, AST, ALT, LDH and bilirubin in treated rats compared to control. No gross pathological and histopathological findings and difference in relative organs weight were observed between control and treated rats. Preliminary phytochemical evaluation shows the presence of various bioactive constituents. Acute toxicity study reveals that LD₅₀ of AECS and MECS is greater than 2000mg/kg. body weight (b. wt) in fasted female rats and can be classified and under category 5. The 28- day repeated oral toxicity study justified that the No Observed Adverse Effect Level (NOAEL) of *Clerodendrum serratum* (CS) is greater than 900mg/kg/b.wt/day/P.O in rats .There were no delayed effects in CS Satellite group. In conclusion CS was found to be non-toxic in tested dose and experimental conditions.

Key words: *Clerodendrum serratum*, Acute toxicity study, 28- day repeated toxicity study.

INTRODUCTION

Clerodendrum serratum (Verbenaceae) is a tropical medicinal plants distributed in the forest of western ghates of india. In Indian system of medicine, that plant is well known as bharangi (Sanskrit) and commonly known as blue glory (English) and Gantu bharangi (Kannada) (Manjunatha BK *et al.*, 2004). As per the traditional claims roots are the potential source of drugs for ailments such as asthma, body ache, bronchitis, fever, cholera dropsy, eye disease, inflammation, malaria,

snake bite, rheumatism, tuberculosis wounds and ulcer (Keshavamurthy KR, 1994).

Despite the use of the plant in traditional, so far no scientific evaluation was carried out on this plant for the toxicity profile preclinically. Our study was therefore undertaken to screen phytochemical constituents and determine the toxicity profile of aqueous and methanolic extracts of aerial parts of *Clerodendrum serratum* (AECS&MECS) on Wistar Albino rats. The acute toxicity and 28-day repeated dose studies may be required to predict the safety and effects of long term exposure of a particular medicinal plant. This study therefore seeks to assess *Clerodendrum serratum* for its toxic effects by seeing body weight and organ weight changes and

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hematological and serum biochemical parameters and changes in histopathology.

MATERIALS AND METHODS

Plant material

The aerial parts of *Clerodendrum serratum* were collected from Tirumala hills, Tirumala, Chittoor Dt, A.P., India. The plant was identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, A.P., India.

Preparation of extract

After shade drying the aerial parts of *Clerodendrum serratum* were blended in to fine powder with a blender and used for the preparation of aqueous and methanolic extracts. The aqueous extract was prepared by cold maceration process for a period of 72 h with occasional stirring. Then the mixture was filtered and the filtrate was collected by removing solvent under reduced pressure (Sharmistha C & Chandra Kalita J, 2012). Methanolic extract was prepared by using soxhlet extractor for 18-20 h. The extract obtained, was concentrated and dried under reduced pressure at controlled temperature (40-50 °C) (Srinivasan R et al., 2007).

Selection of experimental animals

Wistar Albino rats of either sex (130-160 g) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient temperature of 22 ±2 °C and 45-65% relative humidity, with a 12 h light followed by 12 h dark. All the animals were acclimatized for at least 7 days to the laboratory conditions prior to experimentation. Tap water and food pellets were provided ad libitum. Food pellets was withheld overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of Guide for the care and Use of Laboratory animal.

Preliminary phytochemical screening

The phytochemical tests were carried out on the aqueous and methanolic extracts of aerial parts of *Clerodendrum serratum* to determine the bioactive compounds using standard procedures (Raaman N, 2006).

Acute oral toxicity study

The acute oral toxicity study was performed as per the Organisation for Economic and Cooperation and Development (OECD) 423 guide lines. Nine female rats (130-160 g b.wt) were divided in to three groups (3per group) i.e., control and two test groups. Control group received distilled water as vehicle at a dose of 10 ml/kg b.wt while the test groups received an oral dose of 2000mg/kg bwt of AECS and MECS (10 ml/kg b.wt). All

the experimental animals were observed for their mortality and clinical signs of toxicity (general behavior, respiratory patterns, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 30 min, 1, 2 and 4 h and thereafter once a day for 14 days following vehicle, AECS and MECS administration. Body weights were recorded once a week. On 15th day the overnight fasted rats (water allowed) were euthanized using CO₂ euthanasia chamber and subjected to morphological examination of all the major internal organs such as brain, heart, lung, liver, kidney, spleen, adrenals and sex organs. LD₅₀ cut-off value of AECS and MECS was determined in accordance with Globally Harmonized System of Classification and labeling of chemicals (OECD, 2001).

Methodology

A 28-day repeated oral toxicity study was performed according to the OECD guideline, TG 407. In the present study, AECS and MECS were administered at three dose levels i.e., at 100, 300 and 900 mg/kg/day. Both sexes of Wistar albino rats (130-160 g) were divided in to 7 groups with 10 animals (5 males + 5 females) in each. Group I served as control and received distilled water as vehicle orally at a dose of 10 ml/kg b.wt. Remaining 6 groups received AECS and MECS at 100 (Group II &III), 300 (Group IV & V) and 900 (Group VI &VII) mg/kg/day, P.O, respectively (10 ml/kg b.wt. in distilled water), for a period of 28 days. In order to determine the reversibility or recovery from toxic effects, additional satellite groups were preset. Group VIII served as satellite control (received distilled water) Group IX and X served as treatment satellite groups which received AECS and MECS at 900 mg/kg/day, P.O for a period of 28 days. Then the satellite groups were scheduled for follow-up observations for the next 14 days without vehicle or AECS and MECS administration (OECD, 2008).

Observation

All the experimental animals were observed for mortality and morbidity twice a day, till the completion of treatment. Clinical observations were made once daily to detect signs of toxicity. The focus of observation was same as described above for the acute toxicity study. Body weights of the animals were recorded once a week.

Blood analysis

At the end of the stipulated treatment period, the overnight fasted animals were anesthetized, whole blood samples were collected by cardiac puncture for haematological and biochemical analysis. Haematological parameters such as RBC count, haemoglobin (Hb), hematocrit (HCT), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelets, white

blood cell count (WBC) and lymphocytes were analysed by fully automated analyser. Biochemical parameters such as serum glucose, cholesterol, protein, Blood Urea Nitrogen (BUN), creatinine, bilirubin, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) were analysed.

Histopathology

Necropsy was done in all the animals on 29th day. After blood collection all the animals were euthanized for gross pathological examinations of all major internal organs. Organs such as heart, liver, lung, spleen and kidney were collected from all the animals for weighing and calculating relative organ weights and for histopathology.

Statistical analysis

The statistical analysis were carried out by one way ANOVA followed by Dunnett's multiple comparison test for the control and treatment groups using Graph Pad prism 5.0. P value ≤ 0.05 was considered as significance.

RESULTS

Phytochemical analysis

The result of the phytochemical screening of the extracts of *Clerodendrum serratum* is presented in Table 1. The analysis revealed the presence of flavonoids, terpenoids, carbohydrate and sterols in both the extracts. Tannins also present in methanolic extract.

Acute oral toxicity study

There was no treatment related death or signs of toxicity developed in the control, AECS and MECS treated rats through the study. Rubbing of nose and mouth on the floor of the cage and restlessness were the only behavioral signs of toxicity shown by the animals and these disappeared with in 24 h of extracts administration. During the study there were no significant changes in body weights of treated rats compared to control group. Further there were no gross pathological abnormalities in both control and treated rats.

Repeated dose 28-day oral toxicity study

General behavior

There were no noticeable change in the general behavior; treatment related toxicity signs and mortality observed in both sexes of rats treated at 100,300 and 900 mg/kg of both aqueous and methanolic extracts orally for a period of 28 days and in the satellite groups of rats. The final body weights of rats of all groups increased slightly when compared to control groups. The results are depicted in Table 2.

Haemogram

Haematological parameters such as red blood corpuscles, haemoglobin, hematocrit, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration, platelets, white blood corpuscles and lymphocytes were found to be well within the clinical range of rats (Anonymous 1) in the experimental groups which are shown in Table 3.

Biochemical indices

There was a significance increase in cholesterol and protein levels in AECS and MECS treated rats and slightly reduce of alkaline phosphatase, aspartate transaminase, alanine transaminase, bilirubin and lactate dehydrogenase in rats treated with AECS (300 & 900 mg/kg/day) and MECS (300 & 900 mg/kg/day) compared to the control groups. No changes in other biochemical parameters like glucose, blood urea nitrogen and creatinine, observed between control and treated groups the results of which are depicted in Table 4

Organ weights

There were no significant differences in organ and relative organ weights of heart, lung, liver, kidney and spleen recorded between the control, AECS and MECS treated groups and the relative organ weights are illustrated in Table 5.

Histopathology

The macroscopic analysis of the target organs of the treated rats did not show changes in colour and texture when compared with the control group.

DISCUSSION

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (Builders MI *et al.*, 2012). Hence herbal drugs have received greater attention as an alternative to clinical therapy and the demand for these herbal remedies has greatly increased recently. Their utilization is often based in long term clinical experience. Despite the usage of the plants in folklore medicine over ages, only lately has pharmacology and toxicology of these plants begun to receive attention from scientists. Hence, to validate their claimed pharmacological properties and investigate their possible toxicity, preclinical toxicity studies were carried out initially on the aqueous and methanolic extracts of aerial parts of *Clerodendrum serratum* in Wistar Albino rats.

In the present study, during acute toxicity evaluation, there were no mortality and toxicity signs observed at 2000 mg/kg, *Clerodendrum serratum* can be classified under category 5 and LD₅₀ value was greater than 2000 mg/kg in accordance with Globally Harmonized System of Classification and Labeling of

chemicals and this provides us a direct relevant for protecting human and animal health. A 28-day repeated oral toxicity study was performed following OECD test guideline 407 in both male and female Wistar Albino rats. Since examination of clinical signs plays major role in toxicological testing, mortality and morbidity were recorded twice a day throughout the study.

AECS and EECS did not produce any alterations in the feed and water consumption of treated rats that are insignificant when compared to that of control. This reveals that it does not adversely affect the basic metabolic processes of the experimental rats.

The haemopoietic system serves as an important target for toxic chemicals and is a sensitive index for pathological conditions both in humans and animals. In the study, treatment with AECS and MECS did not produce any alteration in haematological parameters (i.e. RBC, haemoglobin, HCT, PCV, MCV, MCH, MCHC, Platelets, WBC and lymphocytes) which indicate that *Clerodendrum Serratum* did not affect blood cells and their production.

In case of animal bodyweights, the extracts treated group showed slightly increase in bodyweight. This suggests *Clerodendrum Serratum* could increase the cholesterol level in blood due to may be the presence of flavonoids, terpenoids, β -sitosterol, γ -sitosterol and campesterol (Mukesh Kr. Singh *et al.*, 2012). Which are the phyto constituents possess male anti- fertility effect in plants (Mamatha Azmeera *et al.*, 2012). Increased level of cholesterol may be due to decreased androgen production,

which results in accumulation of cholesterol in testes and impaired spermatogenesis (Bedwal RS *et al.*, 1994).

There was significant increase in protein levels in AECS (300 & 900 mg/kg/day) MECS (300 & 900 mg/kg/day) treated rats compared to control groups which may be due to its property of increased protein synthesis.

The insignificant difference in urea and creatinine levels between the treated groups and the control group probably suggests that the extract did not interfere with the renal capacity to excrete the metabolite. Indeed creatinine is known as a good indicator of renal function. Any rise in creatinine levels is only observed if there is a marked damage to functional nephrons (Susrutha K *et al.*, 2006). Histopathological slides of kidney structure showed normal structural features suggesting the preserved renal integrity of AECS and MECS treated rats.

Ordinarily, liver cell damage is characterized by a rise in the enzyme levels like AST, ALP, ALT, LDH etc. *Clerodendrum Serratum* did not induce hepatic cellular changes. A rise in serum alkaline phosphatase (ALP) level is usually a characteristic finding in cholestatic liver disease (Builders MI *et al.*, 2012). Insignificant difference between the control and treated rats justifies that no possible cholestasis occurred at dose levels tested.

Histopathological studies of heart, lung, liver, spleen and kidney of the treated rats did not demonstrate significant changes in morphology indicating the protective effect of AECS and MECS on these tissues.

Table 1. Phytochemical screening of extracts of aerial parts of *Clerodendrum serratum*

Phytochemicals	Aqueous extract	Methanolic extract
Alkaloids	-	-
Flavonoids	+	+
Tannins	-	+
Saponins	-	-
Glycosides	-	-
Terpenoids	+	+
Sterols	+	+
Phenols	-	-
Volatile oils	-	-
Carbohydrate	+	+
Proteins and amino acids	-	-

+ VE indicates Present -VE indicates absent

Table 2. Effects of the *Clerodendrum serratum* on bodyweight gain of rats-repeated oral toxicity study

Groups	Dose	Treatment	Body weight in g/week						
			0 day	1st	2nd	3rd	4th	5th	6th
Control	10ml/kg/ P.O	Distilled water	145.22±2.11	145.44±2.22	156.21±2.02	164.25±0.72	175.36±0.54	-	-
C.S treated group	100mg/kg/ P.O	Aq extract	148.33±1.22	145.23±1.10	158.32±1.52	169.12±1.08*	176.22±0.48	-	-
		Me extract	147.93±1.12	148.33±1.13	157.32±1.56	170.10±1.12*	175.39±1.02	-	-
	300mg/kg/ P.O	Aq extract	151.13±1.10	147.23±1.12	156.03±1.65	172.20±1.07*	176.56±1.23	-	-
		Me extract	143.45±1.12	148.54±1.54	157.21±1.56	174.21±1.44*	179.32±1.44	-	-
	900mg/kg/ P.O	Aq extract	141.34±1.71	146.36±1.81	159.32±1.48	179.02±1.08*	180.48±1.24*	-	-
		Me extract	143.22±1.98	154.32±1.97*	160.21±1.54*	178.25±1.14*	179.84±1.11	-	-

Satellite group	Control 900mg/kg/P.O	Distilled water	142.55±1.79	143.55±1.89	155.66±1.87	164.68±1.54	176.54±1.09	189.22±0.43	199.21±0.47*
		Aq extract	146.11±1.74	146.33±1.84	158.88±1.88	167.48±1.44	180.36±1.54*	185.42±1.66	199.33±1.62*
		Me extract	147.24±1.44	147.24±1.98	161.25±1.64*	168.36±1.45*	181.65±1.45*	186.12±1.52	198.32±1.48*

Values are expressed as mean \pm SEM; g - gram; Aq - Aqueous; Me- Methanolic; CS- *Clerodendrum serratum*; (-): Not observed;

Significance with Dunnett's test following one way ANOVA is evaluated as *p < 0.05, **p < 0.01 and ***p < 0.001 vs control group

Table 3a. Effects of the extract of *Clerodendrum serratum* on haematological parameters of rats-repeated oral toxicity study

Groups	Dose	Treatment	Haematological parameter					
			RBC (10 ⁶ /cmm)	Haemoglobin (g/dl)	HCT (%)	PCV (%)	MCV (fl)	MCH (pg)
Control	10ml/kg/P.O	Distilled water	7.12±0.22	14.32±0.54	36.16±2.30	45.32±1.85	48.32±1.52	19.58±1.03
C.S treated group	100mg/kg/P.O	Aq extract	7.42±0.21	13.25±0.52	33.11±2.28*	48.22± 1.88*	48.23± 1.89	19.36±0.98
		Me extract	7.44±0.23	12.57±0.56	36.22±2.12	46.32±1.89	46.38±1.87	20.12±1.03
	300mg/kg/P.O	Aq extract	7.26±0.31	12.84±0.62	35.32±2.47	46.32±1.82	46.22±1.29	19.98±1.05
		Me extract	7.32±0.54	13.14±0.54	33.25±2.36*	45.92±1.90	48.92±1.90	20.36±0.95
	900mg/kg/P.O	Aq extract	6.99±0.21	12.32±0.48	32.55±1.85*	45.32±2.06	44.90±2.36*	21.35±1.06
		Me extract	6.32±0.44	13.70±0.54	36.54±1.98	44.99± 2.11	47.95± 2.21	19.65±0.94
Satellite group	Control 900mg/kg/P.O	Distilled water	7.11±0.54	12.36±0.52	35.68±2.54	47.99±1.89	48.99±1.88	21.36±1.05
		Aq extract	7.25±0.24	13.25±0.55	36.11±1.45	46.99±0.98	46.19±2.08	20.55±1.08
		Me extract	7.33±0.66	14.66±0.66	37.25±1.44	47.96±1.58	47.66±1.52	22.33±1.08*

Table 3b. Effects of the extract of *Clerodendrum serratum* on haematological parameters of rats-repeated oral toxicity study

Groups	Dose	Treatment	Haematological parameter			
			MCHC(g/dl)	Platelet(10 ³ /cmm)	WBC($\times 10^3$ /cmm)	Lymphocyte (%)
Control	10ml/kg/P.O	Distilled water	36.22±1.98	681.50±8.23	9.65±1.32	68.22 \pm 3.12
C.S treated group	100mg/kg/P.O	Aq extract	36.22±2.03	684.50±10.00	10.33±1.66	70.16±3.21
		Me extract	40.32±1.56	687.56±8.36	9.54±1.54	69.23±3.65
	300mg/kg/P.O	Aq extract	36.44±1.88	682.36±6.21	10.54±0.98	72.78±4.23*
		Me extract	38.55±1.54	685.65±6.23	10.66±0.96	68.26±4.32
	900mg/kg/P.O	Aq extract	37.59±1.66	685.66±5.47	9.66±1.66	73.22±4.36*
		Me extract	34.66±1.58	681.70±6.54	10.98±0.98	71.54±3.88
Satellite group	Control 900mg/kg/P.O	Distilled water	41.22±1.58	693.50±9.54*	11.17±1.44	70.68±4.65
		Aq extract	40.36±2.03	697.56±4.22*	12.36±1.54*	69.32±4.21
		Me extract	38.65±1.77	685.63±5.61	9.54±1.36	71.25±3.15*

Values are expressed as mean \pm SEM; Aq -Aqueous; Me- Methanolic; CS- *Clerodendrum serratum*; Significance with Dunnett's test following one way ANOVA is evaluated as *p < 0.05 **p < 0.01 and ***p < 0.001 vs control group. RBC - Red blood cell; HCT - Hematocrit; PCV - Packed cell volume; MCV - Mean corpuscular concentration; MCH - Mean corpuscular haemoglobin; MCHC - Mean corpuscular haemoglobin concentration; WBC - White blood corpuscles.

Table 4a. Effects of the extracts of *Clerodendrum serratum* on biochemical parameter of rats-repeated oral toxicity study

Groups	Dose	Treatment	Bio chemical parameters					
			Glucose (mg/dl)	Cholesterol (mg/dl)	Protein (g/dl)	BUN (mg/dl)	Creatinine (mg/dl)	ALP(mg/dl)
Control	10ml/kg/P.O	Distilled water	105.36 \pm 9.15	92.36±6.32	5.47 \pm 0.65	16.35±1.23	0.74±0.05	122.32±9.23
C.S treated group	100mg/kg/P.O	Aq extract	102.35± 9.54	94.33±6.32	5.55±0.23	14.23±1.25	0.75±0.02	121.22±8.32
		Me extract	103.55±9.36	96.33±5.26*	5.88±0.65	15.24±1.36	0.84±0.03	125.32±7.25
	300mg/kg/P.O	Aq extract	104.65±9.54	106.22±6.21*	5.98±±0.44	14.23±1.45	0.77±0.06	121.65±6.32*
		Me extract	108.55±9.88	105.55±5.32*	7.15±0.23	15.69±1.55	0.69±0.04	116.32±7.44*
	900mg/kg/P.O	Aq extract	110.24±9.36	109.54±4.88*	7.78±0.65	18.23±1.25*	0.81±0.09	110.32±6.25*
		Me extract	106.22±9.54	101.25±5.99*	8.32±0.36*	15.22±1.24	0.76±0.06	108.65±7.10*
Satellite group	Control 900mg/kg/P.O	Distilled water	107.65±9.87	93.24±5.98	5.64±0.25	16.32±1.33	0.75±0.05	123.66±6.55
		Aq extract	109.88±9.55	104.32±6.21*	7.98±0.24	14.23±1.44	0.86±0.06	107.32±6.33*
		Me extract	150.65±9.66	105.66±6.55*	8.23±0.44*	15.88±1.21	0.84±0.02	108.25±7.25*

Table 4b. Effects of the extracts of *Clerodendrum serratum* on biochemical parameter of rats-repeated oral toxicity study

Groups	Dose	Treatment	Bio chemical parameters			
			AST(IU/L)	ALT(IU/L)	LDH(IU/L)	Bilirubin (IU/L)
Control	10ml/kg/P.O	Distilled water	70.36±5.32	72.33±6.32	266.32±5.23	0.45±0.06
C.S treated group	100mg/kg/P.O	Aq extract	65.32±5.54	69.22±5.32	264.23±6.23	0.39±0.06
		Me extract	61.23±6.12*	68.23±5.63	264.45±5.23	0.36±0.05
	300mg/kg/P.O	Aq extract	69.33±6.11	67.62±4.44*	255.45±6.2*	0.30±0.02
		Me extract	64.77±5.55	66.23±5.32	254.32±4.25*	0.31±0.07
	900mg/kg/P.O	Aq extract	60.23±6.23*	62.33±5.26*	261.22±7.25*	0.29±0.09
		Me extract	58.25±6.24*	61.36±5.99*	264.25±8.24	0.28±0.05
Satellite group	Control 900mg/kg/P.O	Distilled water	70.32±6.54	71.12±6.22	261.32±5.21	0.44±0.06
		Aq extract	62.31±5.23*	61.12±6.15*	262.25±6.98	0.27±0.05
		Me extract	59.11±4.99*	60.36±5.55*	263.41±5.22	0.29±0.06

Values are expressed as mean ±SEM; Aq - Aqueous; Me- Methanolic; CS- *Clerodendrum serratum*; Significance with Dunnett's test following one way ANOVA is evaluated as *p < 0.05 **p < 0.01 and ***p < 0.001 vs control group. BUN - Blood urea nitrogen; ALP - Alkaline Phosphatase; AST - Aspartate transaminase; ALT - Alanine transaminase; LDH - Lactate dehydrogenase.

Table 5. Effects of *Clerodendrum serratum* on relative organ weights of rats-repeated oral toxicity study

Groups	Dose	Treatment	Relative organ weight				
			Heart	Liver	Lung	Spleen	Kidney
Control	10ml/kg/P.O	Distilled water	0.48 ±0.01	4.43±0.12	0.49±0.02	0.46±0.01	1.26±0.02
C.S treated group	100mg/kg/P.O	Aq extract	0.45±0.02	4.45±0.13	0.48±0.01	0.44±0.02	1.24±0.05
		Me extract	0.44±0.02	4.54±0.12	0.49±0.02	0.45±0.02	1.08±0.15
	300mg/kg/P.O	Aq extract	0.49±0.02	4.55±0.17	0.48±0.03	0.44±0.02	1.15±0.11
		Me extract	0.47±0.02	4.25±0.18	0.51±0.02	0.43±0.02	1.14±0.06
	900mg/kg/P.O	Aq extract	0.45±0.01	4.38±0.17	0.48±0.01	0.42±0.01	1.25±0.12
		Me extract	0.48±0.01	4.38±0.15	0.47±0.02	0.46±0.02	1.24±0.13
Satellite group	Control 900mg/kg/P.O	Distilled water	0.49±0.02	4.77±0.17	0.48±0.03	0.45±0.02	1.11±0.14
		Aq extract	0.48±0.01	4.62±0.18	0.48±0.01	0.47±0.02	1.21±0.15
		Me extract	0.47±0.02	4.55±0.20	0.51±0.02	0.45±0.02	1.14±1.10

Values are expressed as mean ± SEM; Aq - Aqueous Me- Methanolic; CS- *Clerodendrum serratum*; Significance with Dunnett's test following one way ANOVA is evaluated as *p < 0.05 **p < 0.01 and ***p < 0.001 vs control group.

CONCLUSION

This study has shown the diversity in toxicity as well as the chemical constituents of the aerial parts of *Clerodendrum Serratum* in relation to the extraction solvent. The LD₅₀ value was found to be greater than 2000 mg/kg bwt. With reference to the Globally Harmonized System of Classification and labeling the

chemicals, *Clerodendrum Serratum* can be classified as Category-5. The No Observed Adverse Effect Level (NOAEL) of *Clerodendrum Serratum* was estimated to be greater than 900 mg/kg/day. This study provides the basis for further study on the detailed toxic and pharmacological effects of the extracts of aerial parts of *Clerodendrum Serratum* and their active component(s).

REFERENCES

- Anonymous 1. <http://www.ahu.umn.edu/rar/refvalues.html> (Accessed on 05.11.11).
- Bedwal RS, Edwards MS, Katoch M, Bahuguna A, Dewan R, Histological and biochemical changes in testis of zinc deficient strain of mice. *Indian J Exp Biol*, 32, 1994, 243-247.
- Builders MI, Isichie CO, Aguiyi. JC. Toxicity study of the extracts of *Parkia biglobosa* stem bark in rats. *Br J Pharm Res*, 2, 2012, 1-16.
- Keshavamurthy KR. Medicinal Plants of Karnataka. Karnataka State Forest department 1994, 92.
- Mamatha Azmeera, A. Elumalai, M.Chinna Eswaraiah, Nikhitha Mathangi. An Updated Review on Anti-Fertility Plants- 2012. *Inter. J. Of Pharmacotherapy*, 2(1), 2012, 4-6.
- Manjunatha BK, Krishna V & Pullaiah T. Flora of Dvanagre District: Karnataka, India, Regency publication, New Delhi, India, 2004, 311.
- Mukesh Kr. Singh, Gaurav Khare, Shiv Kr. Iyer, Gotmi Sharwan and D. K. Tripathi. *Clerodendrum serratum*: A clinical approach. *Journal of Applied Pharmaceutical Science*, 2 (2), 2012, 11-15.
- Organization for Economic Co-operation and Development. Guidance Document on Acute Oral Toxicity Testing 423 [R]. Paris: OECD; 2001.
- Organization for Economic Co-operation and Development. OECD 407 Guidelines for the Testing of Chemicals: Repeated Dose 28-day Oral Toxicity Study in Rodents[R]. Paris: OECD; 2008, 5.

- Raaman N. *Phytochemical Techniques*. New Delhi: New India Publishing Agency; 2006, 19-24.
- Sharmista Chakravarthy, Chandra Kalita Jogen. Preliminary phytochemical screening and acute oral toxicity study of the flower of *Phylgacanthus hirsiflorus* Nees in albino mice. *Int Res J Pharm*, 3, 2012, 293-295.
- Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B. Antioxidant activity of *Caesalpinia digyna* root. *J Ethnopharmacology*, 113(2), 2007, 284-91.
- Susrutha K, Satyanarayana S, Srinivas N, Sekhar S, Raja J. Evaluation of blood glucose reducing effects of aqueous extracts of the selected umbelliferous fruits used in culinary practice. *Trop J Pharmaceut Res*, 5, 2006, 613-617.