

## Acute and Sub-Chronic Toxicity Study of *Clerodendron infortunatum* Leaf in Adult Male Albino Mice

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**Abstract:** In the present study, the safety profile of *Clerodendron infortunatum* leaf was evaluated by acute and sub-chronic toxicity study of the methanol extract of *C. infortunatum* leaf (MECI) in Swiss albino mice. In acute toxicity study, MECI up to 2000 mg/kg body weight (b.w.) did not produce any toxic effect or death. In sub-chronic toxicity study, MECI was administered at the single daily dose of 500 mg/kg b.w., i.p. for 28 consecutive days and at the 29<sup>th</sup> day, the hematological, histological, serum and hepatic biochemical parameters were evaluated by sacrificing the animals. No mortality was observed during the course of whole study period. No detectable alterations were found in hematological, biochemical and histological parameters in MECI treated group when compared to vehicle control group after 28 days. The results of the present study therefore indicated that *C. infortunatum* leaf is safe in adult male albino mice demonstrating no noticeable toxicity.

**Key words:** Sub-Chronic Toxicity • *Clerodendron Infortunatum* • Leaf • Biochemical

### INTRODUCTION

*Clerodendron infortunatum* Linn. belonging to the family Verbenaceae, commonly known as *Bhant* in Hindi is a small shrub occurring throughout the plains of India. This plant have been used in Indian folk medicine for the treatment of bronchitis, asthma, fever, burning sensation, diseases of blood, inflammation and epilepsy [1]. Traditionally, the plant is used as an antipyretic and antihelmentic. Leaf of the plant are prescribed for tumour, certain skin diseases and scorpion sting [2]. Previous phytochemical investigation of this plant revealed the presence of alkyl sterols and 2-(3, 4-dehydroxyphenyl) ethanol 1-*o*- $\alpha$ -2 rhamnopyranosyl-(1-3)- $\beta$ -D-(4-*o*-caffeyl) glycopyranoside (acteoside) [3, 4]. Previously the present authors have reported anticancer, anticonvulsant, anti-inflammatory and antihyperglycemic activities of its leaf extract in experimental animal models [5-8]. Still now, there are no reports on toxicity study of this plant. Therefore, the present study was aimed to

investigate the acute and sub-chronic toxicity profile of methanol extract from *Clerodendron infortunatum* leaf (MECI) in adult male Swiss albino mice to establish its safety profile in rodents.

### MATERIALS AND METHODS

**Plant Material:** The plant *Clerodendron infortunatum* Linn. was collected in the month of November 2008 from the forest region of Midnapore, West Bengal, India. The taxonomical identification of the plant was done by the Botanical Survey of India, Shibpur, India. The voucher specimen (PMU-4/JU/2008) has been preserved in Pharmacology Research Laboratory, Jadavpur University, Kolkata for future reference.

**Preparation of Extract:** The leaves of the *Clerodendron infortunatum* was dried under shade and then powered by mechanical grinder to a coarse powder. The powder plant material was extracted with 80% methanol using Soxhlet

extraction apparatus. The solvent was completely removed under reduced pressure and semisolid mass was obtained (Yield 13.5% w/w) stored in a vacuum desiccator for further use. Preliminary phytochemical screening of the plant extract exhibited the presence of flavonoids, tannins and saponins in MECI [9].

**Drugs and Chemicals:** Bovine serum albumin from Sigma Chemical Co., St. Louis, Mo, USA; Trichloroacetic acid (TCA) from Merck Ltd., Mumbai, India; Thiobarbituric acid (TBA), 5,5'-dithio bis-2-nitro benzoic acid (DTNB), Phenazonium methosulphate (PMS), Nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO Research Laboratory, Mumbai, India. Potassium dichromate and glacial acetic acid from Ranbaxy, Mumbai. All the other reagents used were of analytical reagent grade obtained commercially.

**Animals:** Adult male Swiss albino mice weighing 18-25 g were used for the present investigation. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25 ± 2°C with dark/light cycle 14/10 h). They were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to experiment. All experimental procedures described were reviewed and approved by the Jadavpur University Animal Ethical Committee.

**Acute Toxicity:** The acute toxicity of MECI in male Swiss albino mice was studied as reported method [10]. MECI was given to four groups ( $n = 6$ ) of animals at 50, 500, 1500 and 2000 mg/kg body weight, i.p. The treated animals were kept under observation for 3 days, for mortality and general behaviour. No death was observed till the end of the study.

**Sub-Chronic Toxicity:** The adult male Swiss albino mice were divided into two groups containing 6 animals per group. The first group received normal saline (5 ml/kg body weight, i.p.) and the second group received MECI at 500 mg/kg body weight i.p., daily for 28 consecutive days. Food and water intake of animals were observed during this period. Twenty four hours after the last dose (i.e., at the 29<sup>th</sup> day), blood was collected from overnight fasted rats of each group by cardiac puncture for estimation of hematological and serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver biochemical parameters and organ weights.

**Body Weight and Organ Weights:** The body weight of mice of each group were measured just before and 28 days after MECI treatment, respectively. Heart, lung, liver and kidney weights of all mice were measured immediately after post treatment sacrifice.

**Hematological Studies:** Collected EDTA treated blood was used for the estimation of hemoglobin (Hb) content; red blood cell count (RBC) and white blood cell counts (WBC) by standard procedures [11].

**Estimation of Serum Biochemical Parameters:** Collected EDTA treated blood was used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), serum cholesterol and total protein contents by using commercially available reagent kits (Span Diagnostics, Surat, India).

**Estimation of Liver Biochemical Parameters:** Lipid peroxidation i.e., thiobarbituric acid reactive substances (TBARS) was estimated by the previously reported method and expressed as mM/100 g of liver tissue [12]. Reduced glutathione (GSH) was determined by the reported method and was expressed as mg/100 g of liver tissue [13]. Catalase (CAT) activity was assayed according to the method described by standard method and expressed as  $\mu$ moles of H<sub>2</sub>O<sub>2</sub> consumed/min/mg of liver tissue [14].

**Statistical Analysis:** The all experimental data were expressed as mean ± standard error of mean (SEM).

## RESULTS

In acute toxicity study, MECI up to 2000 mg/kg body weight did not produce any signs and symptoms of toxic effects or death. There were no significant changes in body weights and organ weights of mice of MECI treated group (after 28 days) from saline control group (Table 1). No mortality was evident from the experimental results in mice. The food and water intake of MECI treated group was found comparable to the control group without showing significant alteration in body weight and growth rate. From the present study it was seen that there was no significant changes in the number of WBC, RBC and Hemoglobin in the MECI treated group compared to normal control group (Table 2). After 28 days of treatment no significant alterations were observed in all serum

Table 1: Effect of MECI on body weight and weight of major organs in mice

Treatment	Initial body wt (g)	Final body wt (g)	Final Heart wt (g)	Final Lung wt (g)	Final Liver wt (g)	Final Kidney wt (g)
Normal control (0.9% NaCl)	18±1.12	25±1.11	1.51±0.14	1.82±0.19	2.18±0.12	0.66±0.02
MECI (500 mg/kg)	19±0.8	24±1.15	1.48±0.13	1.86±0.18	2.17±0.15	0.69±0.08

Values are expressed as mean ± SEM (n = 6)

Table 2: Effect of MECI on hematological parameters in mice

Treatment	Hemoglobin (g/dl)	RBC (10 <sup>6</sup> cells/ml)	WBC (10 <sup>3</sup> cells/ml)
Normal control (0.9% NaCl)	13.82±1.14	6.76±0.16	3.92±0.42
MECI(500 mg/kg)	13.35±1.42	6.83±0.27	4.12±0.19

Values are expressed as mean ± SEM (n = 6)

Table 3: Effect of MECI on serum biochemical parameters in mice

Treatment	SGOT (IU/dl)	SGPT (IU/dl)	SALP (IU/dl)	Bilirubin (mg/dl)	Cholesterol (mg/dl)	Total protein (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/ml)
Normal control (0.9% NaCl)	42.48±1.27	39.45±1.32	85.37±1.76	0.92±0.13	96.33±6.52	7.54±1.8	41.126±1.12	6.96±1.48	0.96±0.11
MECI(500 mg/kg)	43.76±1.16	38.28±1.26	88.14±1.58	0.93±0.36	95.63±7.84	7.85±1.7	44.52±1.27	7.05±1.32	1.18±0.21

Values are expressed as mean ± SEM (n = 6)

Table 4: Effect of MECI on liver biochemical parameters in mice

Treatment	TBARS (mM/100 g of wet liver tissue)	GSH (mg/100 g of wet liver tissue)	CAT (µmoles of H <sub>2</sub> O <sub>2</sub> consumed/min/mg of wet liver tissue)
Normal control (0.9% NaCl)	1.14±0.6	46.26±1.7	84.36±2.8
MECI(500 mg/kg)	1.16±0.5	43.22±1.9	83.58±2.6

Values are expressed as mean ± SEM (n = 6)

and hepatic biochemical parameters in animals of MECI treated group when compared to normal control group (Tables 3, 4).

## DISCUSSION

The present study was aimed to investigate the possible toxic effects of the methanolic extract of *C. infortunatum* leaf (MECI) in adult male Swiss albino mice. In results of acute toxicity study revealed that MECI may be safe in Swiss albino mice. Various physical, chemical and histological parameters were thoroughly studied in the sub-chronic toxicity study.

The body weights, food and water intakes were found to be unaltered during the 28 days treatment period when compared to control group. Similarly there were no significant changes in different organ weights also. No mortality was observed during this period. Also in the study of hematological parameters there was no alteration of the normal levels of RBC, WBC and hemoglobin count with MECI treated group. Therefore, MECI had no toxic effect on the blood and haematopoietic system.

The serum biochemical parameters were studied to evaluate the possible alterations in hepatic and renal functions influenced by MECI. Biochemical parameters related to hepatic vital functions viz. SGPT, SGOT, SALP, bilirubin, cholesterol contents exhibited no significant alterations as compared with the normal control mice. It is well known that almost all drugs, chemicals and xenobiotics are eliminated through renal excretion hence it was found necessary to estimate the effects of MECI on kidney functions. Serum biochemical parameters related to kidney functions viz. urea, uric acid creatinine and total protein demonstrated no significant differences with respect to control group animals. Therefore, it can be inferred that MECI did not affect the normal hepatic and renal functions on 28 days treatment.

Free radicals or reactive oxygen species (ROS) are regarded to be involved in the pathogenesis of several degenerative diseases and toxic reactions [15]. Antioxidants can retard or stop the uncontrolled generation of ROS, thus help to reduce oxidative stress-induced diseases [16]. In the present study, liver endogenous antioxidant parameters viz. lipid peroxidation

(TBARS), reduced glutathione (GSH) and catalase activity (CAT) were estimated to ascertain the functioning of normal liver endogenous antioxidant defense systems and it was found that no alterations in these parameters took place thereby implying maintenance of normal hepatic non-enzymatic and enzymatic antioxidant mechanisms during MECI treatment.

From the present investigation, it can be concluded that MECI exhibited excellent safety profile in acute and sub-chronic toxicity studies. The present study establishes the reliable safety profile of MECI in adult male Swiss albino mice offering no obvious toxicity.

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