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# Sub-Chronic Toxicity study of Aqueous extract of *Clerodendrum Phlomidis* Leaves

**Gupta Reena*, Duggal Sanjiv, Kapoor Bhupinder**
Lovely School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, India.

**Abstract**

*Clerodendrum phlomidis* Linn. has been traditionally used for treatment of gynecological disturbances and for agricultural uses. It has been used in many Ayurvedic polyherbal formulations as an immunomodulatory agent. Irrespective of its widespread use, no data on sub-chronic toxicity has been described. The present study was designed to access sub-chronic toxicity of aqueous extract of *Clerodendrum phlomidis* leaves.

Aqueous extract of *Clerodendrum phlomidis* leaves was given orally at doses of 200, 400 and 800 mg/kg/day for 90 days for the evaluation of sub-chronic toxicity study. General behavior, mortality, animal body weight, food and water consumption were observed throughout the study period. Hematological, biochemical parameters and histopathological analysis were done at the end of study period.

No mortality and abnormal behavior was observed in rats exposed to all the three dose levels. Highest dose produced significant decrease in the red blood cell, hemoglobin and increase in white blood cell count. Biochemical parameters like triglycerides, bilirubin, creatinine and total proteins were significantly altered at high dose. Histopathological findings revealed architectural changes in the liver and kidneys with high dose.

*Corresponding author, Mailing address:
**Ms. Reena Gupta**
Department of Pharmaceutical Sciences,
Lovely Professional University,
Phagwara (Punjab), India.
Email id: reenaph14@gmail.com

***Key words:***
Sub-chronic toxicity; *Clerodendrum phlomidis*; Herbal medicine; Biochemical parameters; Aqueous extract

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## 1. Introduction

There has been a shift in interest from synthetic to herbal medicines [1]. One of the most frequent problems found in medicinal plants is the absence of clinical, toxicological and pharmacological studies [2]. Herbs are generally considered as dietary supplements and are therefore not subjected to

regulations of safety studies. Generally it is due to the thought that herbs are considered 'Natural' and thus are considered as free from risk but herbal plants and their products are not always safe for medicinal use [3,4,5].

*Clerodendrum phlomidis* Linn. also known as 'Arni' (family- Verbenacae) is a medicinal plant, which is commonly used in Indian Traditional Medicines. It is known to contain various active principles that possess biological activity against a number of diseases. It has been reported that the ethanol extract of leaves possess anti-inflammatory, hepatoprotective, hypoglycemic and anti-arthritic activities [6,7,8,9]. Antipyretic, immunomodulatory and tranquilizer effects have been reported for the methanol extract of the plant [10,11,12]. Agricultural and veterinary uses are also reported for the plant; the leaves are used for protection of stored grains and to control fruit borer, leaves have been used for the treatment of foot and mouth diseases and constipation in cattle's [13]. The plant is widely used in traditional medicine; leaves in treatment of fever, earache, rheumatic problems, opthalmia and hemorrhoids [14,15,16]. The plant has been used for treatment of gynecological disturbances like syphilis, gonorrhea and leucorrhea [17].

Sterols, flavones, flavanones, chalcone, triterpenes and neo-clerodane diterpenoids have been reported from different parts of plant [13]. Phytochemical screening reported the presence of steroids, alkaloids and flavonoids in methanol and ethanol extract of leaves [8,10,12,18].

The present study was conducted to evaluate the safety profile of the aqueous extract of *Clerodendrum phlomidis* leaves after sub-chronic exposure in rats.

## 2. Materials and Methods

### *2.1. Experimental Animals*

The present study was conducted on healthy male and female Albino Wistar rats. The animals were procured from NIPER, Mohali after approval from Institutional Animal Ethical Committee (vide no: 954/ac/06/CPCSEA/10/2). The animals were acclimatized to the laboratory conditions prior to the experiment. The animals were maintained under constant conditions of temperature (23±2 $^{o}$C) and relative humidity of 50-65%. The animals were housed in plastic cages with saw dust in pair of same sex and each cage contained maximum four numbers of animals. Feed and water were provided *ad libitum*.

### *2.2. Plant Material and Extraction*

Fresh *Clerodendrum phlomidis* plant were collected from NIPER, Mohali and identified & authenticated by Dr. S.C. Sinha, Central Council of Research in Ayurveda and Siddha, Regional Research Institute (Ay.) Patna and a herbarium specimen (specimen voucher, RRI/AMP/2010/629) of the plant has been preserved. The collected leaves were shade dried and powdered by using mechanical grinder. 100 g coarse powder was extracted with 1 L of distilled water by the method of continuous hot extraction at 60 $^{o}$C. The extract was concentrated at 40 $^{o}$C using a rotary evaporator (Popular, India) and was dried by allowing it to stand overnight in vacuum oven (Navyug, India) at 30 $^{o}$C. The yield of dried extract was about 17 g per 100 g of powder. The dried extract was stored in desiccators until further use.

### 2.3. *Phytochemical Screening*

Qualitative phytochemical screening of aqueous extract of *Clerodendrum phlomidis* leaves was carried out using standard procedures [19] and it revealed the presence of biologically active ingredients such as alkaloids, phenols, flavonoids, tannins and saponins.

### *2.4. Sub-chronic Toxicity Study*

The sub-chronic toxicity was conducted according to OECD guidelines (Organization for Economic Co-operation and Development, Guideline-408, adopted on 21$^{st}$ September 1998). The animals were randomly

divided into four groups of 8 animals each. Animals of group I served as control and received the vehicle only by gavage (10 ml/kg of body weight) while those of groups II, III and IV were treated daily by gavage with aqueous extract of *Clerodendrum phlomidis* at 200, 400 and 800 mg/kg body weight respectively for 90 days. The body weight, consumption of food and water were measured weekly throughout the study period.

*2.5. Pre-clinical Observation*

General physical condition of each animal was observed during the experimental period. All animals were observed twice daily for mortality. Physical observations were made throughout the study period. Examination included observation of fur, eyes, nose, abdomen and external genitals; occurrence of secretions and excretions, autonomic nervous system activity (e.g. lacrimation, pilorection, respiratory pattern and response to handling).

*2.6. Hematological Analysis*

Hematological analysis was carried out at the end of the study period. Whole blood was collected by retro-orbital bleeding under light ether anesthesia in eppendorffs tubes with EDTA as anticoagulant (1 mg/ml of blood). The blood sample was analyzed for hemoglobin, red blood cells (RBC), white blood cells (WBC) and platelet count in clinical laboratory using cell counter (Lab life Nobel III, India).

*2.7. Clinical Chemistry*

Blood was collected on 91st day by retro-orbital bleeding under light ether anesthesia in eppendorffs tubes without anticoagulant; serum was obtained by centrifugation at 3000 rpm for 10 minutes. Serum glucose, cholesterol, triglycerides (TGs), high density lipoproteins (HDL), low density lipoproteins (LDL), alanine amino transferase (ALT), aspartate amino transferase (AST), total proteins, bilirubin (total and direct), alkaline phosphatase, urea, uric acid and creatinine levels were measured by biochemical assay kits (Erba Mannheim, Transasia Bio-Medical Ltd. Baddi, India) using auto analyzer (Photometer 5010 V5+, Nicholas Piramal India Pvt. Ltd, Mumbai, India).

*2.8. Histopathology*

The animals of all the groups were sacrificed after the study period and the high and low dose group animals were subjected to gross and histopathological examination. The kidney, heart and liver were examined for histopathological changes. The relative organ weight of the kidney, heart, liver, adrenal gland and brain was calculated as (organ/body weight) x 100 % [20].

*2.9. Statistical Analysis*

The experimental results have been expressed as the mean ± S.E.M. Significant differences were determined using student t-test and differences were considered significant at $p<0.05$, $p<0.02$.

## 3. Results

*3.1. Pre-clinical Observations*

The animals from control and treatment groups were removed from their cages and examined once weekly during the 13 weeks of study period for any sign of toxic effect. No unusual change in the behavior, ataxia and sign of intoxication were observed during the 13 weeks of study period and all the animals survived until scheduled necropsy. The general condition of the animals suggested no harmful effect of the aqueous extract of leaves.

*3.2. Body Weight*

Control group animals gained weight throughout the study period (13% in male and 14 % in female animals). Decrease in body weight was seen in both medium and high dose group animal but this decrease in body weight was not statistically significant (Fig.1).

*3.3. Food and Water consumption:*

Decrease in food and water consumption was observed in the 1st week of treatment in control and treatment group animals. Food and water consumption was continuously decreased in high and medium dose group animals throughout the study period; however the consumption was increased in control group animals (Fig. 2 and Fig 3).

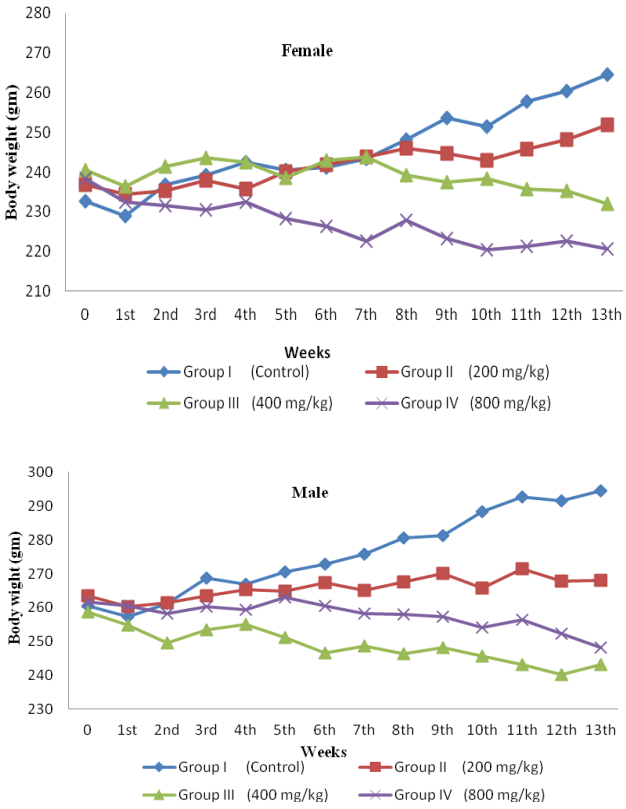
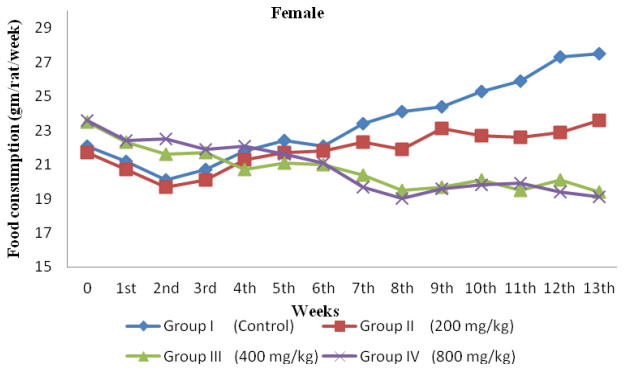



**Fig. 1.** Effect of aqueous extract of *Clerodendrum phlomidis* leaves on body weight of animals.



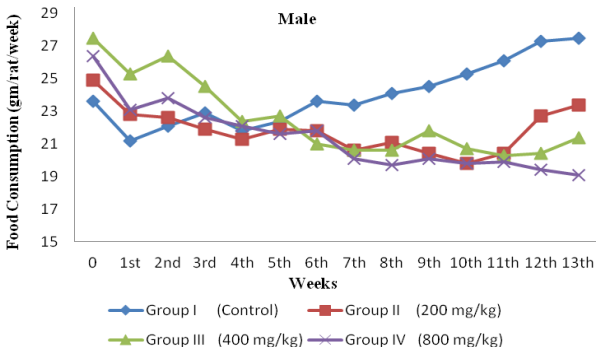




**Fig. 2.** Effect of aqueous extract of *Clerodendrum phlomidis* leaves on food consumption of animals.

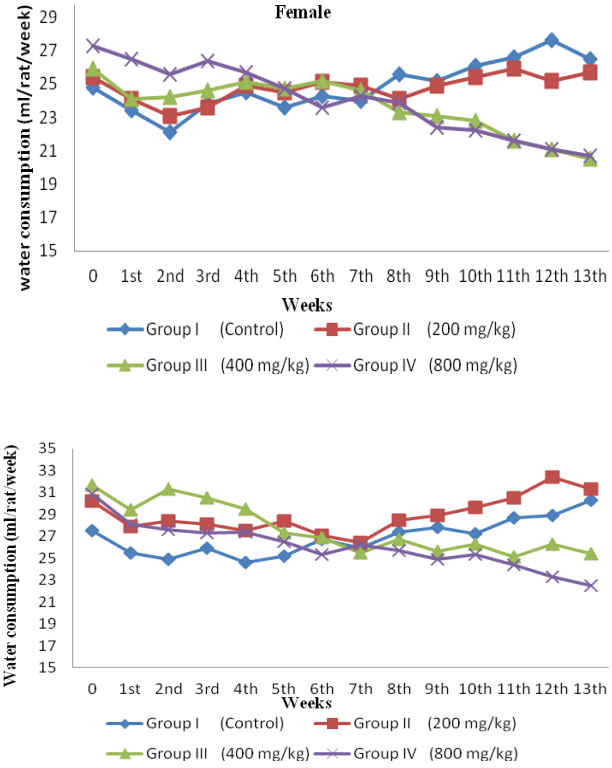



**Fig. 3.** Effect of aqueous extract of *Clerodendrum phlomidis* leaves on water consumption of animals.

*3.4. Hematological Analysis*

There was a significant change in HGB, RBC and WBC count in high dose group animals when compared with control group animals. RBC count and HGB level were significantly decreased in male animals; whereas WBC count was increased significantly in both male and female animals of high dose group (Table 1).

**Table 1:** Effect of aqueous extract of *Clerodendrum phlomidis* leaves on hematological parameters of animals.

| Groups | Dose (mg/kg p.o.) | Haemoglobin (gm/dl) | Red blood cells ($10^6$/µl) | White blood cells ($10^3$/µl) | Platelets ($10^3$/µl) |
|---|---|---|---|---|---|
| *Female* | | | | | |
| Group I | Control | 13.92±0.34 | 7.12±0.13 | 8.32±0.23 | 332.01±0.65 |
| Group II | 200 mg/kg | 14.12±0.47 | 7.05±0.21 | 9.21±0.45 | 295.01±0.44 |
| Group III | 400 mg/kg | 13.10±0.33 | 8.01±0.41 | 8.74±0.31 | 333.05±0.34 |
| Group IV | 800 mg/kg | 13.20±0.44 | 7.23±0.51 | 12.21±0.42* | 360.23±0.21 |
| *Male* | | | | | |
| Group I | Control | 14.56±0.64 | 8.94±0.31 | 9.56±0.16 | 371.04±0.11 |
| Group II | 200 mg/kg | 15.94±0.31 | 9.64±0.23 | 9.41±0.45 | 392.02±0.24 |
| Group III | 400 mg/kg | 15.31±0.24 | 9.23±0.43 | 9.72±0.23 | 420.72±0.34 |
| Group IV | 800 mg/kg | 11.95±0.21** | 6.12±0.56* | 13.63±0.17* | 400.13±0.54 |

*p<0.05, **p<0.02, control vs low, intermediate and high dose. Values are mean ± SEM, n = 8.

*3.5. Clinical chemistry*

Significant decrease in blood glucose level was seen in high dose group compared with control group at P<0.05. The triglyceride and VLDL levels were significantly (P<0.05) increased in the male animals of high dose group whereas LDL level was significantly decreased in high dose group. There was no significant alteration in lipid profile of female animals except increase in LDL level (Table 2).

Significant increase in serum urea and creatinine level was observed in both male and female animals of the high dose group animals when compared to the control group animals. No significant change was observed in the uric acid level in all the treatment group animals.

There was no significant difference in the alkaline phosphatase levels in all the treatment group animals compared to control group animals. Significant change was observed in total and direct bilirubin count of male animals at high dose (800 mg/kg) group animals when compared with control animals.

**Table 2:** Effect of aqueous extract of *Clerodendrum phlomidis* leaves on glucose and lipid profile of animals.

| Groups | Dose (mg/kg p.o.) | Glucose (mg/dl) | Triglyceride (mg/dl) | Cholesterol (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) |
|---|---|---|---|---|---|---|---|
| *Female* | | | | | | | |
| Group I | Control | 118.21±1.21 | 58.34±2.84 | 73.28±3.21 | 28.23±1.37 | 33.39±1.43 | 11.66±0.37 |
| Group II | 200 mg/kg | 115.31±1.11 | 56.94±2.09 | 72.45±5.32 | 27.54±1.54 | 33.52±1.54 | 11.38±0.29 |
| Group III | 400 mg/kg | 117.42±1.21 | 59.28±1.43 | 71.53±4.04 | 29.31±1.29 | 30.36±1.41 | 11.89±1.15 |
| Group IV | 800 mg/kg | 96.03±1.41* | 60.12±1.31 | 72.44±5.42 | 31.43±1.39 | 31.27±2.31* | 12.11±1.11 |
| *Male* | | | | | | | |
| Group I | Control | 122.32±1.41 | 62.78±2.84 | 77.02±4.54 | 32.65±1.47 | 31.82±1.56 | 12.55±0.58 |
| Group II | 200 mg/kg | 125.32±1.29 | 64.65±2.09 | 76.12±5.34 | 33.21±1.49 | 29.98±1.38 | 12.93±0.34 |
| Group III | 400 mg/kg | 120.23±1.26 | 63.26±1.43 | 77.76±2.04 | 31.23±1.36 | 33.88±2.12 | 12.65±1.13 |
| Group IV | 800 mg/kg | 102.65.±1.16* | 76.65±1.31* | 78.56±5.22 | 31.56±1.49 | 31.67±2.25* | 15.33±1.22* |

*p<0.05, control vs low, intermediate and high dose. Values are mean ± SEM, n = 8.

**Table 3:** Effect of aqueous extract of *Clerodendrum phlomidis* leaves on kidney function test of animals.

| Groups | Dose (mg/kg p.o.) | Urea (mg/dl) | Uric acid (mg/dl) | Creatinine (mg/dl) | Urea: Creatinine |
|---|---|---|---|---|---|
| *Female* | | | | | |
| Group I | Control | 20.32±1.12 | 1.02±0.11 | 0.71±0.02 | 28.61±2.21 |
| Group II | 200 mg/kg | 18.76±1.15 | 1.01±0.12 | 0.74±0.01 | 25.35±1.11 |
| Group III | 400 mg/kg | 19.12±1.21 | 1.13±0.17 | 0.75±0.05 | 25.49±2.44 |
| Group IV | 800 mg/kg | 24.24±1.62* | 1.09±0.20 | 1.25±0.02* | 19.39±2.34 |
| *Male* | | | | | |
| Group I | Control | 22.74±1.11 | 1.12±0.15 | 0.75±0.01 | 30.32±2.11 |
| Group II | 200 mg/kg | 22.21±1.14 | 1.09±0.16 | 0.82±0.05 | 27.08±1.21 |
| Group III | 400 mg/kg | 23.14±1.20 | 1.21±0.12 | 0.79±0.03 | 29.29±2.27 |
| Group IV | 800 mg/kg | 28.56±1.48* | 1.14±0.16 | 1.33±0.07* | 21.47±2.56 |

*p<0.001, control vs low, intermediate and high dose. Values are mean ± SEM, n = 8.

**Table 4:** Effect of aqueous extract of *Clerodendrum phlomidis leaves* extract on liver function test of animals.

| Groups | Dose (mg/kg p.o.) | AST (IU/l) | ALT (IU/l) | Total protein (g/dl) | Total bilirubin (mg/dl) | Direct bilirubin (mg/dl) | Alkaline phosphatase (IU/l) | AST:ALT |
|---|---|---|---|---|---|---|---|---|
| *Female* | | | | | | | | |
| Group I | Control | 109.13±2.38 | 71.23±6.43 | 5.15±0.56 | 0.12±0.01 | 0.09±0.02 | 71.43±7.34 | 1.53±0.45 |
| Group II | 200 mg/kg | 114.23±3.20 | 76.20±5.23 | 7.10±0.23 | 0.11±0.07 | 0.12±0.09 | 73.34±5.31 | 1.49±0.23 |
| Group III | 400 mg/kg | 100.65±5.24 | 65.23±5.22 | 7.00±0.42 | 0.12±0.02 | 0.11±0.08 | 76.56±5.17 | 1.54±0.13 |
| Group IV | 800 mg/kg | 101.23±4.21 | 71.69±5.45 | 7.20±0.17 | 0.21±0.02 | 0.19±0.06 | 72.56±6.77 | 1.41±0.65 |
| *Male* | | | | | | | | |
| Group I | Control | 113.21±1.21 | 79.21±6.43 | 7.01±0.35 | 0.16±0.02 | 0.13±0.07 | 85.32±5.76 | 1.43±0.76 |
| Group II | 200 mg/kg | 120.33±7.12 | 82.34±6.70 | 7.18±0.20 | 0.19±0.05 | 0.12±0.06 | 77.56±6.39 | 1.46±0.22 |
| Group III | 400 mg/kg | 108.43±6.43 | 71.67±3.45 | 7.16±0.43 | 0.18±0.02 | 0.15±0.04 | 78.67±4.78 | 1.51±0.35 |
| Group IV | 800 mg/kg | 109.24±5.32 | 77.40±6.34 | 8.04±0.20** | 0.25±0.03** | 0.20±0.02** | 76.98±5.33 | 1.41±0.21 |

**p<0.02, control vs low, intermediate and high dose. Values are mean ± SEM, n = 8.

*3.6. Relative organ weight*

The oral administration of aqueous extract of *Clerodendrum phlomidis* over 90 days did not produce any significant change in the relative weight of the organs (i.e. kidney, heart, liver, adrenal gland and brain) in the high dose treatment group when compared with the control group animals (Table 5).

**Table 5:** Effect of aqueous extract of *Clerodendrum phlomidis* leaves on relative organ weights of animals.

| Groups | Dose (mg/kg p.o.) | Relative organ weight (%) | | | | |
|---|---|---|---|---|---|---|
| | | Kidney | Heart | Liver | Adrenal gland | Brain |
| *Female* | | | | | | |
| Group I | Control | 0.60±0.18 | 0.31±0.15 | 2.49±0.11 | 0.025±0.11 | 0.50±0.12 |
| Group IV | 800 mg/kg | 0.64±0.21 | 0.32±0.18 | 2.50±0.15 | 0.023±0.15 | 0.51±0.19 |
| *Male* | | | | | | |
| Group I | Control | 0.64±0.13 | 0.39±0.17 | 2.57±0.16 | 0.026±0.16 | 0.55±0.15 |
| Group IV | 800 mg/kg | 0.70±0.12 | 0.36±0.21 | 2.64±0.14 | 0.025±0.14 | 0.60±0.13 |

Values are mean ± SEM, n = 8.

*3.7. Histopathological Analysis*

Histological examination of liver section of male animals treated with *Clerodendrum phlomidis* extract (800mg/Kg) showed some abnormal findings. There were only mild lymphocytes and plasma cells in the portal tract. No congestion was observed in the sinusoids. Central vein was intact and findings did not show necrosis in the liver.

Mild focal degenerative changes in the form of inflammatory infiltration were seen in the histopathological examination of kidneys of high dose group treated male animals. The architectural features of glomeruli were normal. No lesions were found the kidneys

No remarkable structural alterations were seen in the histopathology of heart in high dose (800 mg/kg) and control group. (Fig. 4-6)

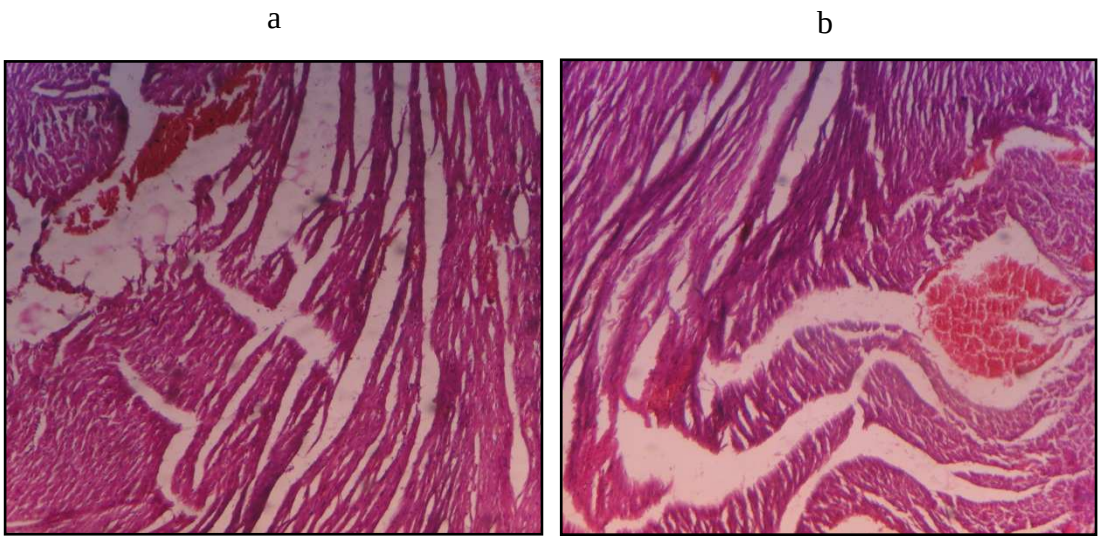



**Fig. 4.** Effect of aqueous extract of *Clerodendrum phlomidis* leaves on the microscopy of heart.

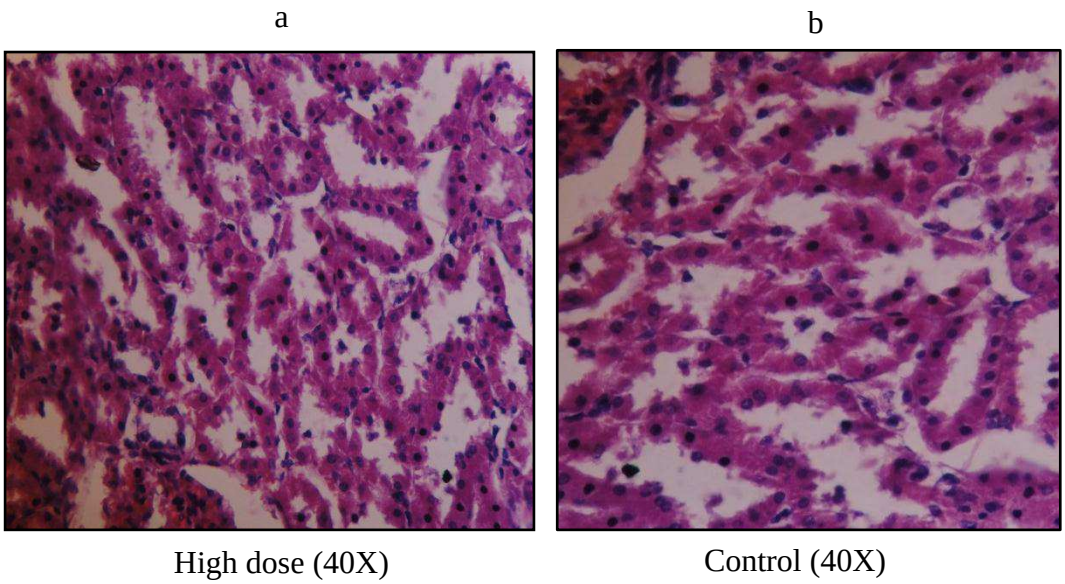



**Fig. 5.** Effect of aqueous extract of *Clerodendrum phlomidis* leaves on the microscopy of kidney.

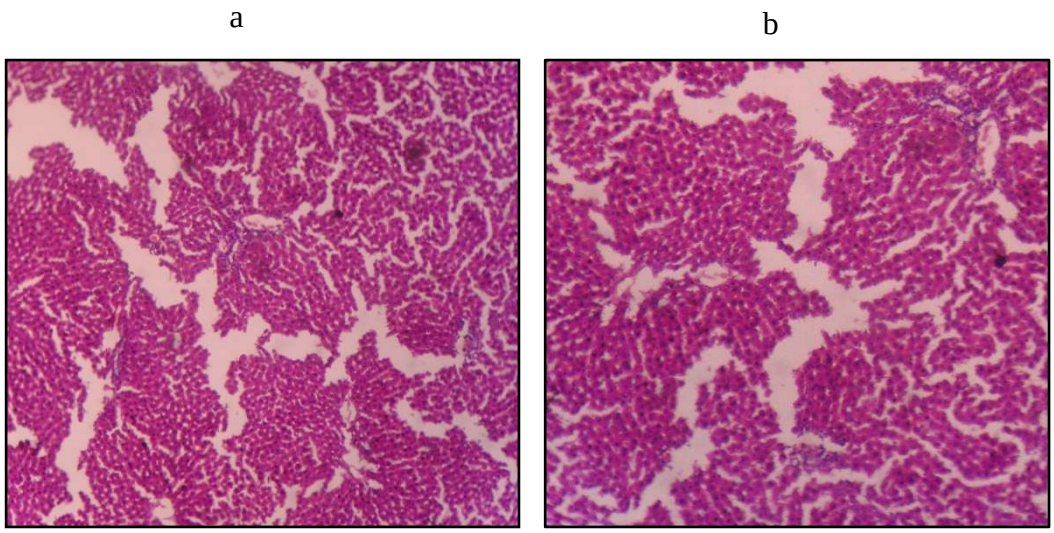



**Fig. 6.** Effect of aqueous extract of *Clerodendrum phlomidis* leaves on the microscopy of liver.

## 4. Discussion

From the Vedic period, *Clerodendrum phlomidis (*Arni) is an important plant with therapeutic uses like, in obesity and inflammation [21]. Traditionally the leaves of the plant have been used in mental disturbances, gynaecological problems, in opthalmia and as a bitter tonic [22,23,24,25]. This plant has been used over long time period; no information is available regarding safety following repeated exposure.

The present study demonstrated the safety profile of aqueous extract of *Clerodendrum phlomidis* leaves in experimental animals. General behavior and body weight are one of the critical parameters for the evaluation of first sign of toxicity [26]. In the present study, the treatment with aqueous extract of *Clerodendrum phlomidis* leaves at medium (400 mg/Kg) and high dose (800 mg/Kg) for 90 days decreased the body weight of animals in comparison to control and low dose group and it may be co-related with the decrease in food consumption of animals.

Hematopoietic system is one of the most sensitive targets for toxic compound and important index of physiological and pathological status; blood profile usually gives vital information on the response of the body to the injury or stress [27,28]. Significant decrease in red blood cell count and hemoglobin was observed in male animals treated with high dose. Significant increase in WBC count was observed in both male and female animals of high dose group this may be attributed to the toxic effect of repeated administration of the plant extract at high dose (800 mg/Kg). Daily oral administration of aqueous extract of *Clerodendrum phlomidis* leaves for 90 days did not produced any significant change in platelet count in all the treatment and control group animals.

Anti-diabetic activity of *Clerodendrum phlomidis* leaves has been reported [8]; in the present investigation high dose of extract produced significant decrease in blood glucose level.

Liver and kidneys play significant roles in metabolic activities of body. Liver is the major organ involved in drug metabolism and kidneys are the site for drug reabsorption and excretion [29]. The increase in levels of AST and ALT in serum is associated with liver toxicity. The transaminases are used as biomarkers for predicting possible toxicity [30]; in the present investigation there was no

significant change in the serum ALT and AST levels in all treatment group animals. Bilirubin is a breakdown product of hemoglobin and is associated with hepatic diseases like jaundice, ineffective erythropoiesis and hepatic cholestasis [31]. Significant increase in serum total protein, total bilirubin and direct bilirubin was observed in the male animals of high dose group. These finding can be correlated with the presence of mild lymphocytes and plasma cells in the portal tract of liver of high dose group animals.

High serum levels of cholesterol and triglycerides associated with cardiovascular diseases [32]. The triglyceride and VLDL levels were significantly increased in the male animals of high dose group but these changes were toxicologically irrelevant as the data was within normal laboratory range. The increased levels of TG & VLDL are associated with atherogenic risk [33]. The cholesterol level was not significantly changed when compared to control group animals.

Urea, uric acid and creatinine are considered as important markers for kidney dysfunction [27,34]. The increase in serum urea and creatinine levels observed in high dose group is associated with kidney dysfunction and it can be correlated with the histopathological findings of kidney at high dose group that showed mild degenerative changes in the kidney. Although these parameters were increased in female animals of high dose group but no microscopical changes were seen in histopathology. No significant change was observed in the uric acid level in the all the treatment groups.

In conclusion, no pre-clinical symptoms of toxicity were observed on oral administration of aqueous extract of *Clerodendrum phlomidis* leaves. Mild to moderate significant changes were observed in liver and kidney biochemical markers that were also co-related with histopathological findings. This study provides valuable data on the sub-chronic toxicity profile of *Clerodendrum phlomidis* that can be useful in chronic toxicity study and clinical study of this valuable medicinal plant.

**Acknowledgement**

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