Int J Biochem Mol Biol 2022;13(4):40-48
www.ijbmb.org /ISSN:2152-4114/IJBMB0137420

Original Article

Acute and chronic toxicity studies on ethanolic leaf extracts of *Clerodendrum viscosum* and *Leucas indica* in Swiss albino mice

Rajan Chandrashekar[1], Mohandas Rai[1], Bhuvanesh Sukhlal Kalal[1,2,3]

[1]Department of Pharmacology, A. J. Institute of Medical Sciences and Research Centre, Mangaluru 575004, Karnataka, India; [2]A. J. Research Centre, A. J. Institute of Medical Sciences & Research Centre, Mangaluru 575004, Karnataka, India; [3]Department of Pharmacology and Nutritional Sciences, College of Medicine, University of Kentucky, Lexington, Kentucky 40536, USA

Received July 16, 2021; Accepted August 16, 2022; Epub August 20, 2022; Published August 30, 2022

Abstract: Background and objectives: To evaluate the safe dose range of *Clerodendrum viscosum (C. viscosum)* and *Leucas indica (L. indica)* ethanolic leaf extracts of acute and chronic oral toxicity study in Swiss Albino mice. Materials and methods: The Organization for Economic Co-operation and Development guideline was used for the toxicity studies. C. viscosum and L. indica plant extract were administered orally in a single dose of 2000 mg/kg, and general behavior, adverse effects, and mortality were studied for 72 h. For the chronic toxicity study, both plant extracts were administered orally to a separate set of animals at 300 mg/kg doses for 90 days. Animals body weight was taken out, blood and gastric juice were collected for biochemical parameters, and vital organs were collected for histopathological studies after sacrificing test and control group animals. Results: Both in acute and chronic toxicity assay, there was no significant alteration in body weight, physical signs, symptoms, hematological, biochemical parameters, and body organ weights compared to the normal group. The liver, kidney, and stomach histology did not show any drug-induced lesion. Conclusions: The result indicates that the oral administration of *C. viscosum* and *L. indica* ethanolic plant extract did not cause any toxicological effects. Hence it could be regarded as a safe natural product for therapeutic use.

Keywords: Toxicity, Swiss Albino mice, safety study, *Clerodendrum viscosum*, *Leucas indica,* ethanolic plant extract, toxicology

Introduction

Herbal medicine is one of the elements of complementary and alternative medicine, its increasing gaining favor around the world. According to popular belief, herbal remedies are “natural” and thus intrinsically safe. However, if used incorrectly, their effects can be extremely powerful and perhaps fatal, and their use as a substitute for traditional treatments may be ineffectual. Hepatotoxicity of primary ingredients, contamination of preparations by heavy metals or bacteria, unpleasant reactions owing to age, and genetic and concurrent disease characteristics of the user have all been linked to hazardous consequences [1, 2].

Clerodendrum viscosum (*C. viscosum*) is a well know medicinal plant in Ayurveda where extracts from different plant parts have been used to treat other illnesses such as skin diseases, cancer, blood disorders, etc [1]. Despite of that very few compounds have been isolated from *C. viscosum* and there are limited toxicological studies. Similarly, *Leucas indica (L. indica)*, another indigenous plant mentioned in Indian system of medicine used as analgesic, anti-inflammatory, antipyretic, antiparasitic and sedative drug [3-5]. Although this plant has tremendous potential against human diseases practically, there are no reports of its acute and or chronic toxicity screenings.

Although pharmacological effects of *C. viscosum* and *L. indica* plant extract are useful, a comprehensive awareness of its toxicity potential is still lacking.

Therefore, the current study is aimed to evaluate the acute toxicity of aqueous ethanolic

extract of *C. viscosum* and *L. indica* leaves in the animal model.

Materials and methods

Plant material

The whole plants of *C. viscosum* and *L. indica* were collected in the month of June to August 2010 from the Manjanady village (13.0036° N, 75.0654° E) located in Mangaluru region of Karnataka, India. Professor Krishna Kumar G, Department of Applied Botany, Mangalore University, authenticated the plants (voucher 6/10/2010).

Extraction

Fresh leaves were separated from the whole plant and were cleaned, dried in the shade, grinded mechanically, and a fine powder was prepared. One kilogram of air-dried powdered was loaded in Soxhlet extractor in different phases and extracted using 90% ethanol as solvent for 36 h. The extract was air dried and concentrated (40-50°C) using a rotary evaporator. The dried extracted material was weighed, and the percentage yield was calculated using the formula; *% Extraction = $[(m_1-m_2)/m_1]$ × 100,* where m_1 served as sample mass (gms) prior to extraction and m_2 as the post-extraction sample mass (gms). The extract was suspended in 1% gum acacia and was used in these experimental studies.

Laboratory animals

The study was approved by the Institutional Animal Ethical Committee (IAEC), Yenepoya (Deemed to be University), Mangaluru, India (Ref. No.06/05/2010). The animal experiments were carried as per 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, New Delhi, India.

After one week of acclimatization in the animal house, young, healthy non-pregnant female Swiss-albino mice weighing 25-30 gm were used in this study. The animals were housed in polypropylene cages with sterile paddy husk as beddings and maintained with relative humidity 55±5%, room temperature 25±2°C, and 12/12 h light/dark cycle. Standard rodent diet and purified water were fed to the animals [6, 7].

Acute oral toxicity assay

For all the experiments, animals were fasted for food for 12 h (overnight) and allowed free access to water. The next day, they were randomly allocated into three groups with 6 animals in each and administered oral treatment as follows. On day 1, Group I received 1% gum acacia (3 ml/kg); Group II received an ethanolic extract of leaves of *C. viscosum* (2000 mg/kg); and Group III received an ethanolic extract of leaves of *L. indica* (2000 mg/kg). Prior to dose administration, the body weight of each animal was measured. Food was provided after one hour of dosing. All the animals were observed twice daily for the next 13 days, making a total of 14 days.

Preclinical observation: Animals were weighed, and their body weights were recorded regularly. The monitoring of general physical conditions such as observation of fur, eyes, nose, abdomen, and external genitals, including any occurrence of secretions and also autonomic nervous system activity (e.g., lacrimation, piloerection, respiratory pattern, and response to handling) was observed in each animal to determine the onset of peripheral observable toxic reactions throughout the experimental period [8].

Biochemical analysis: On 14th day, animals were sacrificed by high-dose chloroform anesthesia, and blood samples were collected by cardiac puncture for liver and renal function tests. The blood sample was collected in the plain tube (red stopper), centrifuged at 3000 rpm for 10 mins to obtain serum, and stored at -20°C until assay. After collecting the blood, the stomach was opened out and gastric juice was collected, and its volume was measured. Gastric function tests were done measurement gastric content and determination of gastric pH, total acidity, and free acidity. All the vital organs were separated for histopathological studies.

Serum samples were subjected for liver function tests which included estimation of total bilirubin, direct bilirubin, indirect bilirubin, total proteins, albumin, globulin, serum glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), gamma-glutamyltransferase gamma-glutamyltranspeptidase (Gama GT)

Table 1. Effects of EELCV and EELLI on the organ to body weight indices in mice

Organs	Control (1% Gum acacia) 3 ml/kg	*C. viscosum* 2000 mg/kg	*L. indica* 2000 mg/kg
Liver	7.81±0.14	8.03±0.12	7.96±0.12
Kidney	1.49±0.05	1.45±0.02	1.39±0.02
Stomach	1.34±0.01	1.35±0.01	1.35±0.01

Values are presented as mean ± S.E.M., N = 6; EELCV, ethanolic extract of the leaves of *Clerodendrum viscosum*; EELLI, ethanolic extract of the leaves of *Leucas indica*; organ-to-body weight index was calculated by formula (organ weight × 100)/body weight. The observations are mean ± S.E.M. P>0.05 Not Significant in extract treated and vehicle-treated groups (ANOVA followed by Dunnett's multiple comparison test).

and renal function test which included estimation of blood urea and serum creatinine which were measured by biochemical assay kits.

To determine total acidity, an aliquoted of 1 mL gastric juice was diluted with 1 mL distilled water and pipette out in a 50 ml conical flask. After adding two drops of phenolphthalein indicator, the mixture was titrated with NaOH (0.01 N) until a permanent pink color was established. The volume of NaOH utilized in titration was noted, and the total acidity was calculated using the formula: n × 0.01 × 36.45 × 1000 (meq/l); where n is the volume of NaOH consumed. The free acidity test was done using the same procedure and formula except for the Topfer's reagent as an indicator.

The liver, kidney, and stomach's relative organ weight (ROW) was calculated as follows: ROW = (absolute organ weight/mice body weight) × 100%. The harvested organs were immediately fixed with 10% formalin; after being embedded in paraffin wax, thin sections of 5 mm were made and stained with hematoxylin and eosin. The slides were observed under a light microscope, and tissue images were captured.

Chronic oral toxicity assay

The animals were grouped into three containing three animals in each, and drugs were administered orally. Group I served as the control, received the vehicle (1% Gum acacia) only by gavage (3 ml/kg of body weight); Group II and Group III received an ethanolic extract of leaves of *C. viscosum* and *L. indica* at a dose of 300 mg/kg body weight. All the groups received once-daily administration of the drugs 90 days. All the evaluation was carried out in the same manner as done for acute toxicity study.

Statistical analysis

Experimental data were presented as mean ± Standard error of the mean (S.E.M.), and one-way ANOVA followed by Dunnett's multiple comparison tests was done. The *p*-value less than 0.05 compared to the control was considered statistically significant.

Results

The Ethanolic Extract of Leaves of *C. viscosum* was a green sticky mass and *L. indica* was a light brown mass. The % yield obtained from *C. viscosum* and *L. indica* were 12.5% w/w and 15.5% w/w with respect to dried leaves.

Behavioral pattern and body weight

The general physical condition of the animals suggested no harmful effect of *C. viscosum* and *L. indica* ethanolic extract at the dose of 200 mg/kg throughout the experimental period. The limit test with a dose of 2000 mg/kg body weights of *C. viscosum* and *L. indica* group leaf extract did not show any mortality.

Organ to body weight index

All the animal's vital organs did not show any sign of lesion. Organ to body weight index (**Table 1**) also did not have any significant variation (P>0.05) among extract-treated and vehicle-treated groups.

Biochemical analysis

The ethanolic extract of *C. viscosum* and *L. indica* had no significant difference in all the gastric function tests for measuring gastric content and determination of gastric pH, total acidity and free acidity when compared with the vehicle control group (**Table 2**).

The liver function tests were normal in both control and *C. viscosum* and *L. indica* treated animals as summarized in **Table 3**. The alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) were significant (P<0.01 and <0.05) decreased in acute toxicity potential of hematological profile. No significant changes were observed in serum urea and serum creati-

Table 2. Effect of EELCV and EELLI on gastric function test recorded during acute toxicity studies in female Swiss Albino Mice

Tests	Control (1% Gum acacia) 3 ml/kg	*C. viscosum* 2000 mg/kg	*L. indica* 2000 mg/kg
Gastric content (ml/100 gm)	4.45±0.13	3.85±0.30	4.15±0.06
pH of the Gastric content	3.81±0.07	4.22±0.16	4.09±0.11
Total acidity of the Gastric content (eq/L)	5753.89±190.97	5420.63±57.75	5316±78.51
Free acidity of the Gastric content (meq/L)	4677.75±113.20	4361.85±183.86	4355.77±77.46

Values are presented as mean ± S.E.M., N = 6; EELCV, ethanolic extract of the leaves of *Clerodendrum viscosum*; EELLI, ethanolic extract of the leaves of *Leucas indica*; S.E.M.: Standard error of the mean.

Table 3. Hematological examination related to liver function test recorded in acute toxicity studies of EELCV and EELLI at the dose of 2000 mg/kg in female Swiss Albino Mice

Test	Control (Mean ± S.E.M.)	*C. viscosum* (Mean ± S.E.M.)	*L. indica* (Mean ± S.E.M.)
Total Bilirubin (mgs/dl)	0.40±0.03	0.45±0.02	0.44±0.01
Direct Bilirubin (mgs/dl)	0.16±0.00	0.16±0.00	0.20±0.01
Indirect Bilirubin (mgs/dl)	0.24±0.01	0.23±0.01	0.25±0.03
SGOT (IU/L)	52.33±1.33	50.83±1.42	54.16±1.07
SGPT (IU/L)	65.33±3.30	73.16±1.70	71.33±1.43
alkaline phosphatase (IU/L)	151.6±3.39	76.82±2.56**	79.33±1.33**
Total Proteins (gms/dl)	7.28±0.25	7.26±0.27	7.73±0.09
Albumin (gms/dl)	4.03±0.14	3.65±0.19	3.68±0.12
Globulin (gms/dl)	3.88±0.16	3.83±0.15	3.95±0.11
Albumin to globulin ratio	0.80±0.03	0.78±0.01	0.81±0.01
Gamma-glutamyl transferase (U/L)	22.66±1.05	20±0.81*	20.33±1.40*

*P<0.05 and **P<0.01 when compared with the vehicle control group. The observations are mean ± S.E.M. P>0.05-Not Significant, P<0.01-Highly significant as compared to control (ANOVA followed by Dunnett's multiple comparison tests) EELCV-Ethanolic Extract of the leaves of *Clerodendrum viscosum*, EELLI-Ethanolic Extract of the leaves of *Leucas indica*, LFT-Liver Function Test, SGOT-serum glutamic oxaloacetic transaminase, SGPT-serum glutamic pyruvic transaminase, A.L.P.-Alkaline Phosphatase, Gama GT-Gamma-glutamyltransferase or gamma-glutamyltranspeptidase, mg/dL-milligrams per deciliters, IU/L-International unit per liters, gms/dl-grams per deciliters, U/L-unit per liters. S.E.M.: Standard error of the mean.

Table 4. Hematological examination related to renal function tests recorded in acute toxicity studies of EELCV and EELLI at the dose of 2000 mg/kg in female Swiss Albino Mice

Test	Vehicle control group	Acute Toxicity Group	
	Control (Mean ± S.E.M.)	*C. viscosum* (Mean ± S.E.M.)	*L. indica* (Mean ± S.E.M.)
Serum Urea *(mg/dl)*	44.33±1.54	43.66±1.40	43.66±1.78
Serum Creatinine *(mg/dl)*	1.21±0.09	1.05±0.10	1.3±0.19

Values are presented as mean ± S.E.M., N = 6; EELCV = ethanolic extract of the leaves of *Clerodendrum viscosum*; EELLI = ethanolic extract of the leaves of *Leucas indica*; organ-to-body weight index = (organ weight × 100)/body weight.

nine levels of control and treated groups (**Table 4**).

Acute toxicity studies of reveals that there were no abnormal findings in histopathological examination in liver, kidney, and stomach of *C. viscosum* and *L. indica* ethanolic extract treated animals in comparison to control animals (**Figure 1**).

Chronic oral toxicity assay

Similarly, in chronic toxicity studies, the general condition of the animals suggested no harmful effect of *C. viscosum* and *L. indica* ethanolic extract at the dose of 300 mg/kg when administered for 90 days. No changes were seen in the body weight and organ weights (liver, kidney, and stomach) when compared between

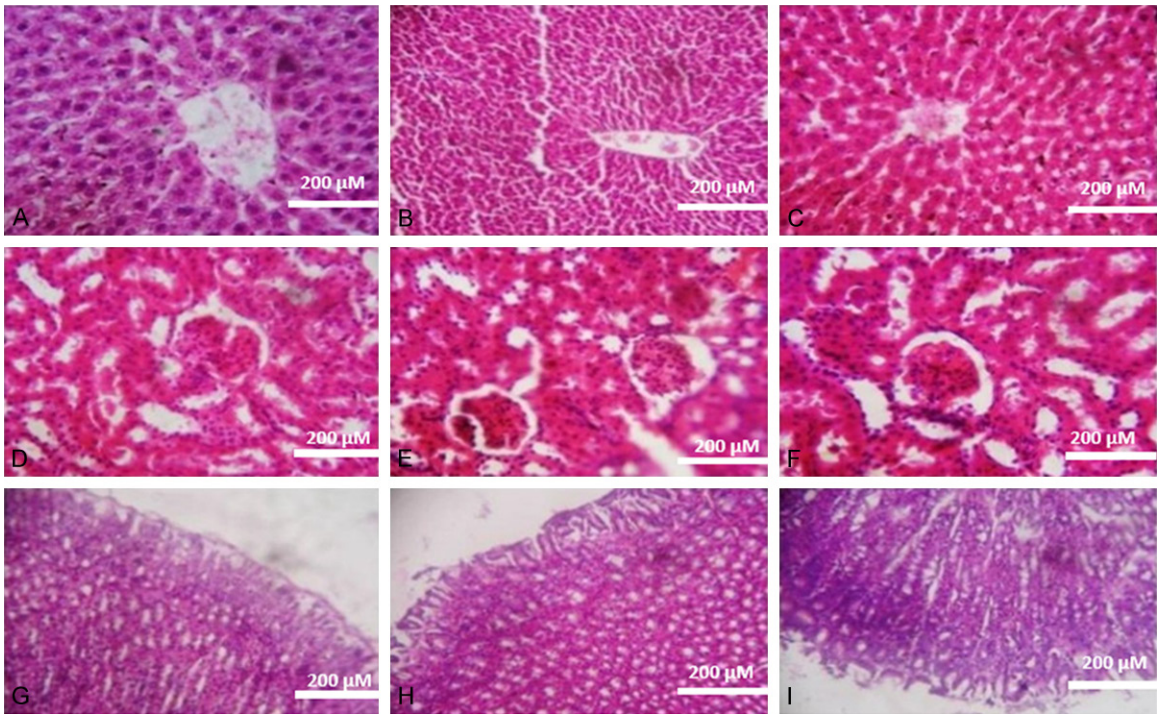

Figure 1. Histopathology of control and ethanol extract treated groups at a limit dose (2000 mg/kg body weight). Histology of liver, kidney, and stomach in vehicle control (1% Gum acacia 3 ml/kg) mice (A, D and G); ethanolic extract of leaves of *Clerodendrum viscosum* animals treated mice (B, E and H); and ethanolic extract of leaves of *Leucas indica* treated mice (C, F and I).

Table 5. Effects of chronic EELCV and EELLI treatment on body weight and the organ to body weight indices in mice

		Control (1% Gum acacia) 3 ml/kg	*C. viscosum* 300 mg/kg	*L. indica* 300 mg/kg
Body weight	Day 0	183.83±5.13	184.26±4.56	175.18±3.94
	Day 90	186.2±3.13	181.2±2.71	177.4±1.62
Organs weight	Liver	8.6±0.29	8.73±0.21	8.46±0.32
	Kidney	1.43±0.11	1.4±0.10	1.4±0.05
	Stomach	1.38±0.00	1.4±0.01	1.39±0.00

Values are presented as mean ± S.E.M., N = 3; organ-to-body weight index was calculated by formula (organ weight × 100)/body weight. S.E.M.: Standard error of the mean. P>0.05 Not Significant in extract treated and vehicle-treated groups (ANOVA followed by Dunnett's multiple comparison test).

the groups (**Table 5**). Biochemical and histopathological examination reveals no abnormality with respect to the liver, renal, and gastric function test in acute toxicity studies (**Table 6**). The present chronic toxicity study reveals that *C. viscosum* and *L. indica* did not produce any toxicity signs related to biochemical parameters like (total bilirubin, direct bilirubin, indirect bilirubin, SGOT, SGPT, ALP, total proteins, albumin, globulin, A/G Ratio, and gamma GT) (**Tables 7**, **8**) and also in histopathology studies of liver, kidney, and stomach (**Figure 2**).

Discussion

The safety and integrity of therapeutic drugs are critical to the pharmaceutical industry, and patient care [9]. Herbal drugs generally are considered safe in contrast to the synthetic drugs that are regarded as unsafe to humans and environment [10]. Though there is huge data available on chemical and biological characterization of plant products, proven scientific studies on the toxicity and adverse effect of these plant products are needed. Both *C. viscosum* and *L. indica* plants have valuable pharmacological effects; the comprehensive awareness about its toxicity potential has not been studied till date. Therefore, the present research evaluated the acute and chronic effects of an ethanol leaf extract of *C. viscosum* and *L. indica* plant. The animal toxicity was done on mice instead of

Table 6. Effect of EELCV and EELLI on gastric function test recorded during chronic toxicity studies in female Swiss Albino Mice

Tests	Control (1% Gum acacia) 3 ml/kg	*C. viscosum* 2000 mg/kg	*L. indica* 2000 mg/kg
Gastric content (ml/100 gm)	5.01±0.32	4.21±0.19	4.16±0.25
pH of the Gastric content	3.81±0.07	4.22±0.16	4.09±0.11
Total acidity of the Gastric content (meq/L)	5528.50±370.24	5440.12±139.28	5303.47±127.23
Free acidity of the Gastric content (meq/L)	4391.22±130.66	4342.87±138.14	4276.8±141.07

Table 7. Hematological examination related to liver function test recorded in chronic toxicity studies of EELCV and EELLI at the dose of 300 mg/kg in female Swiss Albino Mice

Test	Control (Mean ± S.E.M.)	*C. viscosum* (Mean ± S.E.M.)	*L. indica* (Mean ± S.E.M.)
Total Bilirubin (mgs/dl)	0.37±0.01	0.38±0.01	0.39±0.02
Direct Bilirubin (mgs/dl)	0.16±0.006	0.17±0.008	0.16±0.008
Indirect Bilirubin (mgs/dl)	0.20±0.01	0.21±0.01	0.23±0.02
SGOT (IU/L)	205.83±5.6	201.83±5.06	201±7.09
SGPT (IU/L)	69±4.03	66.33±2.92	66±3.06
Alkaline phosphatase (IU/L)	212.66±4.63	163.16±2.08*	167.5±10.33*
Total Proteins (gms/dl)	7.21±0.12	7.06±0.14	7.16±0.13
Albumin (gms/dl)	3.33±0.07	3.23±0.07	3.26±0.08
Globulin (gms/dl)	3.91±0.10	3.83±0.13	3.9±0.12
Albumin to globulin ratio	0.85±0.02	0.84±0.03	0.84±0.03
Gamma-glutamyl transferase (U/L)	22.66±1.25	22±1.00	20.16±1.22

*P<0.05 when compared with the vehicle control group. The observations are mean ± S.E.M. P>0.05-Not Significant as compared to control (ANOVA followed by Dunnett's multiple comparison tests), LFT-Liver Function Test, SGOT-serum glutamic oxaloacetic transaminase, SGPT-serum glutamic pyruvic transaminase, A.L.P.-Alkaline Phosphatase, Gama GT-Gamma-glutamyltransferase or gamma-glutamyltranspeptidase, mg/dL-milligrams per deciliters, IU/L-International unit per liters, gms/dl-grams per deciliters, U/L-unit per liters. S.E.M.: Standard error of the mean.

Table 8. Hematological examination related to renal function tests recorded in chronic toxicity studies of EELCV and EELLI at the dose of 2000 mg/kg in female Swiss Albino Mice

Test	Vehicle control group	Chronic toxicity group	
	Control (3 ml/kg) Mean ± S.E.M.	*C. viscosum* (300 mg/kg) Mean ± S.E.M.	*L. indica* (300 mg/kg) Mean ± S.E.M.
Serum Urea *(mg/dl)*	42.14±1.49	40±1.34	41.5±1.76
Serum Creatinine *(mg/dl)*	1.06±0.04	0.96±0.04	0.91±0.04

Values are presented as mean ± S.E.M., N = 6; EELCV = ethanolic extract of the leaves of *Clerodendrum viscosum*; EELLI = ethanolic extract of the leaves of *Leucas indica*; organ-to-body weight index = (organ weight × 100)/body weight.

rats. It is because toxicity data collected from mice were mainly predicted with the toxic effects in human beings [11].

Indian traditional medicinal systems like Ayurveda, Siddha, and Unani have been used to treat a wide range of diseases since ancient times. Despite of advancement of modern medicine, herbal drugs contributed significantly to the health care system [10, 12]. These drugs are gaining popularity as WHO encourages the use of traditional systems of medicine if possible, implant them into the mainstream of health care services [13]. Indian Council of Medical Research has made substantial research investments in medicinal plants for better drugs and remedies for various diseases [14]. Herbal medicines have played an important role in managing various chronic diseases, attracting clinical researchers to use them as alternatives to allopathic pharmaceutical drugs [15].

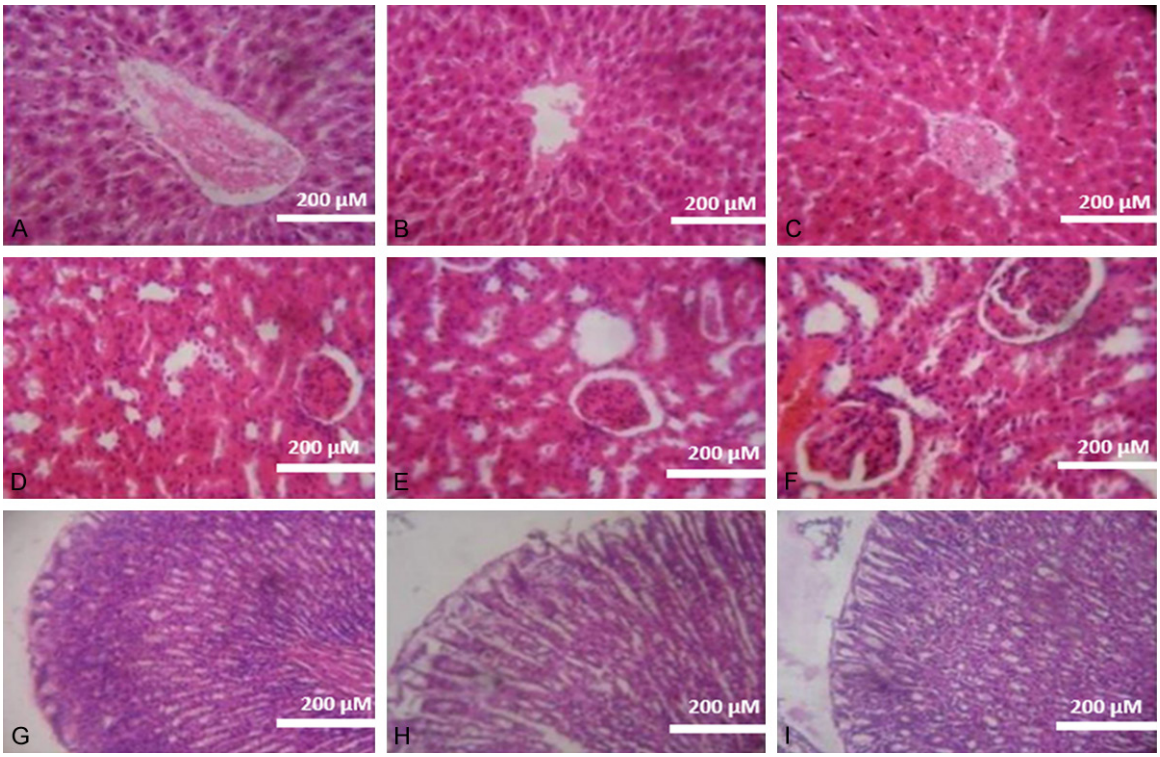

Figure 2. Histopathology of control and ethanol extract treated groups at continuous dose (300 mg/kg body weight) for 28 days. Histology of liver (A-C), kidney (D-F) and stomach (G-I) in vehicle control (1% Gum acacia 3 ml/kg) mice (A, D and G); ethanolic extract of leaves of *Clerodendrum viscosum* animals treated mice (B, E and H); and ethanolic extract of leaves of *Leucas indica* treated mice (C, F and I).

India is an ancient civilization with a rich repository of medicinal plants. Ayurveda's traditional medicinal system developed in India and has been practiced for centuries [16]. Modern drugs have limited availability and usually associated side effects, therefore, researcher encourages usage of traditional medicine [17]. Increasing population, excessive treatment costs and the emergence of drug-resistant to synthetic medicines have increased the popularity of alternative medicines, contributing to countries economic value [18-20].

The leaves of *C. viscosum* belong to the family Verbenaceae/Lamiaceae/Labiatae and *L. India* belongs to family Lamiaceae/Labiatae possess anti-inflammatory and analgesic possibly due to the presence of phytosterols, triterpenes, alkaloids, glycosides, saponins and most probably due to the presence of phenolic compounds and tannins as shown by a phytochemical screening of *C. viscosum* and *L. indica* in this study [1, 21]. These purified compounds and standardized plant extracts provide unlimited opportunities for new therapeutics drugs [6].

Early identification of the potential for substances to cause hepatotoxicity and nephrotoxicity is vital for human wellbeing risk appraisal [22]. The liver is frequently getting affected with chemical-induced injuries and a few factors, including drug effects effectively add to the liver's vulnerability. Since most xenobiotics enter the body through oral route, passes through the gastrointestinal tract, and then carry to the liver, this organ is the most commonly exposed to their assault [23-25]. The subsequent explanation is that the biotransformation of synthetic substances in the body occurs in the liver [26]. The majority of time, biotransformation prompts the development of a particle that is no more or, at any rate, less naturally dynamic, more polar, and water-dissolvable, subsequently more effectively discharged from the body; but at times, the metabolic movement of the liver produces metabolites as well. Without a doubt, the biotransformation of synthetic substances into reactive metabolites has a critical occasion for nephrotoxicity. The nephrotoxic metabolites might be created locally by the activity of P450s in the kidney or they can be delivered in the liver or in

different organs and transported into the kidney through foundational dissemination [27]. The high renal bloodstream and the weighty centralizations of excretory items, getting to the re-assimilation of water from the rounded liquid, are further significant elements in the kidney's weakness to xenobiotics [28].

Acute toxicity and chronic toxicity studies were dose as per Diener et al. and Stallard et al., which served as indicators described in preclinical observation [28, 29]. Most commonly, herbal drugs are administered orally, and hence gastric acid profile was evaluated in this study as both *C. viscosum* and *L. indica* have been used as analgesic, antipyretic and anti-inflammatory agents and commonly used conventional preparations related NSAIDs carry the risk of increasing gastric acidity which limits its use in a susceptible individual. Orally administered drugs usually undergo metabolism in the liver and are excreted in urine via kidney; hence liver function and renal function test was employed in this study to know major organ toxicity.

Biochemical, hematological, and histopathology examinations did not show significant changes in control and treated animals, suggesting that a dose level of 2000 mg/kg (of *C. viscosum,* and *L. indica*) is safer. The pharmaceutical drug or compound with oral LD_{50} more than 1000 mg/kg bw and the oral dose of 1000 mg/kg could be considered low toxic and safe [30, 31]. Toxic outcomes of drugs can be visualized directly by observing the vital body organs [32]. Further, the test with chronic done of 300 mg/kg bw for 28 days of oral toxicity study also did not have any clinical sign of toxicity, and no mortality was recorded in test mice. These results indicating that the repeated dose of *C. viscosum* and *L. indica* ethanol leaf extract have no adverse effect.

Conclusions

In this study, acute toxicity studies (2000 mg/kg) were administered on a single day and observed for 14 days. Chronic toxicity studies (300 mg/kg when administered for 90 days) of *Clerodendrum viscosum* and *Leucas indica* ethanolic leaf extracts did not showed any unusual changes during preclinical observation. Physical, biochemical, and histopathological examination also reveals no abnormality when compared between the groups. Hence, study supports the use of *Clerodendrum viscosum* and *Leucas indica* ethanolic leaf extracts as an alternative system of medicine for the biological and pharmacological screening of the same.

Acknowledgements

The authors would like to thank the A. J. Institute of Medical Sciences and Research Centre for providing facilities and fund for this project.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bhuvanesh Sukhlal Kalal, A. J. Research Centre, A. J. Institute of Medical Sciences and Research Centre, Nh-66 Kuntikan, Mangaluru 575004, Karnataka, India. Tel: +919739309942; ORCID: 0000-0002-2560-3778; Fax: +91824222; E-mail: bhuvanesh611@gmail.com; Bhuvanesh.Kalal@uky.edu

References

[1] Nandi S and Lyndem LM. Clerodendrum viscosum: traditional uses, pharmacological activities and phytochemical constituents. Nat Prod Res 2016; 30: 497-506.

[2] Bateman J, Chapman RD and Simpson D. Possible toxicity of herbal remedies. Scott Med J 1998; 43: 7-15.

[3] Gogoi D, Arora N, Kalita B, Sarma R, Islam T, Ghosh SS, Devi R and Mukherjee AK. Anticoagulant mechanism, pharmacological activity, and assessment of preclinical safety of a novel fibrin(ogen)olytic serine protease from leaves of Leucas indica. Sci Rep 2018; 8: 6210.

[4] Das SN, Patro VJ and Dinda SC. A review: ethnobotanical survey of genus Leucas. Pharmacogn Rev 2012; 6: 100-106.

[5] Ramalingam RBK, Madhavi BB, Nath AR and David B. Pharmacognostical, phytochemical and anthelmintic evaluation of Leucas indica (L). Pharmacognosy Journal 2010; 2: 317-323.

[6] Shetty R, Rai M, Chandrashekar R and Kalal BS. Diabetogenic effect of gluten in Wistar albino rats: a preliminary preclinical screening. Med Pharm Rep 2020; 93: 47-52.

[7] Kalal BS, Modi PK, Upadhya D, Saha P, Prasad TSK and Pai VR. Inhibition of bone morphogenetic proteins signaling suppresses metastasis melanoma: a proteomics approach. Am J Transl Res 2021; 13: 11081-11093.

[8] Sibley K, Chen J, Koetzner L, Mendes O, Kimzey A, Lansita J and Boulos RA. A 14-day repeat dose oral gavage range-finding study of a first-

in-class CDI investigational antibiotic, in rats. Sci Rep 2019; 9: 158.
[9] Alshammari TM. Drug safety: the concept, inception and its importance in patients' health. Saudi Pharm J 2016; 24: 405-412.
[10] Karimi A, Majlesi M and Rafieian-Kopaei M. Herbal versus synthetic drugs; beliefs and facts. J Nephropharmacol 2015; 4: 27-30.
[11] Kratchman J, Wang B and Gray G. Which is most sensitive? Assessing responses of mice and rats in toxicity bioassays. J Toxicol Environ Health A 2018; 81: 173-183.
[12] Mosihuzzaman M. Herbal medicine in healthcare–an overview. Nat Prod Commun 2012; 7: 807-812.
[13] Ozorio P. World Health Organization encourages traditional medicine in the third world. Dev Dir 1979; 2: 16.
[14] Tandon N and Yadav SS. Contributions of Indian Council of Medical Research (ICMR) in the area of medicinal plants/traditional medicine. J Ethnopharmacol 2017; 197: 39-45.
[15] Leonti M and Casu L. Traditional medicines and globalization: current and future perspectives in ethnopharmacology. Front Pharmacol 2013; 4: 92.
[16] Mukherjee PK, Nema NK, Venkatesh P and Debnath PK. Changing scenario for promotion and development of Ayurveda–way forward. J Ethnopharmacol 2012; 143: 424-434.
[17] Sarmukaddam S, Chopra A and Tillu G. Efficacy and safety of Ayurvedic medicines: recommending equivalence trial design and proposing safety index. Int J Ayurveda Res 2010; 1: 175-180.
[18] Sher H, Aldosari A, Ali A and de Boer HJ. Economic benefits of high value medicinal plants to Pakistani communities: an analysis of current practice and potential. J Ethnobiol Ethnomed 2014; 10: 71.
[19] Sen S and Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: importance, challenges and future. J Tradit Complement Med 2017; 7: 234-244.
[20] Kalal BS, Pai VR, Behera SK and Somashekarappa HM. HDAC2 inhibitor valproic acid increases radiation sensitivity of drug-resistant melanoma cells. Med Sci (Basel) 2019; 7: 51.
[21] Rahmatullah M, Jahan R, Azam FM, Hossan S, Mollik MA and Rahman T. Folk medicinal uses of Verbenaceae family plants in Bangladesh. Afr J Tradit Complement Altern Med 2011; 8: 53-65.
[22] Galeano D, Li S, Gerstein M and Paccanaro A. Predicting the frequencies of drug side effects. Nat Commun 2020; 11: 4575.
[23] Shashikumara S, Purushotham K, Darshan CL and Kalal BS. Characterization of antidepressant activity of Saraca asoca flower (Roxb.) Wilde in mice subjected to acute restraint stress. Am J Transl Res 2022; 14: 5014-5023.
[24] Andrade RJ, Chalasani N, Bjornsson ES, Suzuki A, Kullak-Ublick GA, Watkins PB, Devarbhavi H, Merz M, Lucena MI, Kaplowitz N and Aithal GP. Drug-induced liver injury. Nat Rev Dis Primers 2019; 5: 58.
[25] Kalal BS, Pai VR and Upadhya D. Valproic acid reduces tumor cell survival and proliferation with inhibitors of downstream molecules of epidermal growth factor receptor pathway. J Pharmacol Pharmacother 2018; 9: 11-16.
[26] Phang-Lyn S and Llerena VA. Biochemistry, Biotransformation. In: editors. StatPearls. Treasure Island (FL): 2022. p.
[27] Liu S, Yao Y, Lu S, Aldous K, Ding X, Mei C and Gu J. The role of renal proximal tubule P450 enzymes in chloroform-induced nephrotoxicity: utility of renal specific P450 reductase knockout mouse models. Toxicol Appl Pharmacol 2013; 272: 230-237.
[28] Hall AM, Trepiccione F and Unwin RJ. Drug toxicity in the proximal tubule: new models, methods and mechanisms. Pediatr Nephrol 2022; 37: 973-982.
[29] Stallard N and Whitehead A. A statistical evaluation of the fixed dose procedure. Altern Lab Anim 2004; 32 Suppl 2: 13-21.
[30] Erhirhie EO, Ihekwereme CP and Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdiscip Toxicol 2018; 11: 5-12.
[31] Adeneye AA and Olagunju IA. Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of Carica papaya Linn. in Wistar rats. Biology and Medicine 2009; 1: 1-10.
[32] Kalal BS, Fathima F, Pai VR, Sanjeev G, Krishna CM and Upadhya D. Inhibition of ERK1/2 or AKT activity equally enhances radiation sensitization in B16F10 cells. World J Oncol 2018; 9: 21-28.