doi.org/10.36721/PJPS.2023.36.2.REG.491-500.1

Safety assessment of ethanolic extract of *Canarium strictum* Roxb. leaves: Acute and subchronic toxicity studies

Rajkumar Selvam[1], Sathish Kumar Perumal[1], Latha Subbiah[1], Madepalli Byrappa Gowdu Viswanathan[2] and Selvamani Palanisamy[1*]

[1]Department of Pharmaceutical Technology, Centre for Excellence in Nanobio Translational Research, Anna University, Bharathidasan Institute of Technology Campus, Tiruchirappalli Tamil Nadu, India

[2] Department of Botany, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Abstract: *Canarium strictum* Roxb. (Burseraceae) is a tree distributed in India, China and Thailand. In traditional Ayurvedic medicine, it is used to treat asthma, rheumatism, blood impurities, syphilis, fever, epilepsy and cough. Toxicological information is currently unavailable warrants present research. Ethanol leaf extract obtained by soxhlet extraction was used to investigate its toxicity. The acute toxicity data showed ethanolic leaf extract is safe up to 2000mg/kg dose in female albino mice. There were no behavioral or physiological alterations or gross clinical abnormalities. The ethanolic leaf extract was administered orally to Wistar rats (n=5) of both sexes at a dose of 300, 600 and 1200mg/kg/d for 90 days during the investigation of sub-chronic toxicity. There were no treatment-related deleterious effects on general behavior, body weight, relative organ weight, biochemical and hematological parameters in the sub-chronic trial when evaluated daily/weekly. Organ histopathology revealed no significant abnormalities. Additionally, the ethanolic leaf extract improved rats' cholesterol and metabolic profiles. There is no apparent harm with ethanolic leaf extract treatment for 13 weeks, unless the dosage is quite high. Thus, it implies that the leaves are safer to use as a traditional medicine remedy for a variety of conditions in a wide dose range.

Keywords: *Canarium strictum* roxb.; burseraceae; acute toxicity; sub-chronic toxicity

INTRODUCTION

Before the advent of modern medicine, people all over the world had relied on traditional herbal remedies to maintain their health. But modern health care providers are very much concerned about the safety, quality and efficacy of such traditional herbal-based medicines (Jin *et al*., 2018). In spite of this concern, several traditional natural formulations and herbal mixtures were very commonly used in most of the underdeveloped countries to treat a variety of illnesses. Traditional medicine is now considered as an alternative to biomedicine, mainstream medicine, conventional medicine and western medicine in order to compensate some perceived shortcomings in these medicinal systems (Zhu *et al*., 2002).

Medicinal plants are known for their long-term use, one could expect that the bioactive chemicals extracted from such plants are generally considered to be safe for both animals and humans. Nearly 80% of 122 plant-derived drugs are of ethnopharmacological origin (Fabricant and Farnsworth, 2001). Treatment of several diseases including diabetes, cancer, renal impairment, autoimmunity or immunosuppression (AIDS), heart disease and neurological problems can benefit from the use of herbs specified and practiced in Ayurvedic remedies and other traditional medicine systems. Modern treatments have a limited or no success rate or substantial adverse-effects in many cases (Sarker, 2014). The WHO (2019) encouraged member countries to adopt national policies tailored to their specific situations, taking into account that the most frequent type of traditional medication with the usage of medicinal plants around the world. Prior to human use, toxicological evaluations of herbal medicines and dose validation standards are needed to evaluate and understand the associated toxic effects (Ribeiro *et al*., 2019). Therefore, in order to ensure that medicinal herbs are safe for regular usage, they must be thoroughly tested for toxicity.

Black Dammar, *Canarium strictum* Roxb. is a tree species in the family of Burseraceae and is widely distributed in southern parts of India, Sikkim to China (Yunnan) and northern parts of Thailand (Meena *et al*., 2012). Its resin is used along with resins/products of other species of Burseraceae to prepare traditional Ayurvedic remedy of Raladhupa. Plaster and ointment substitutes for Burgundy pitch are made from gum resin (Suruse *et al*., 2010). Decoction or powder of the resin is used to treat asthma, rheumatism, syphilis, cough, blood impurities, fever, different poisons, epilepsy, chronic skin ailments, hernia and hemorrhage (Nadkarni, 1965; Defilipps and Krupnick, 2018). Allergic and pruritic reactions due to exposure with the caterpillar larvae hairs were treated with fresh or molted resin (Hinge *et al*., 1965). The essential oil obtained contains steroids and triterpenoids which has antibacterial and anti-inflammatory properties (Muthuswamy, 2013; Tahir *et al*., 2021). Mogana *et al*. (2013) reported scopoletin (7-hydroxy-6-methoxy

**Corresponding author:* e-mail: selvamani@aubit.edu.in

coumarin) that regulates and increases dopamine concentrations in humans. After numerous studies and widespread use in Ayurvedic medicines, no study has been reported on the toxicological profile of the leaf extract of *Canarium strictum* Roxb. Thus, the purpose of this study was to evaluate the sub-chronic and acute toxic effects of ECSL in mice and rats after oral treatment following OECD rules (OECD, 2002, 2008, 2018) and Good Laboratory Practices for the testing of chemical compounds (2008).

MATERIALS AND METHODS

Authentication of plant material

Botanists and taxonomists at the Jawaharlal Nehru Tropical Botanical Garden & Research Institute, Palode, Thiruvananthapuram, Kerala, India authenticated the fresh specimen of *Canarium strictum* Roxb. (Family: Burseraceae) leaves further verified by Dr. Rajeev Kumar Singh of the Botanical Survey of India [Voucher specimen number: BSI/SRC/5/23/2017/Tech/3203].

Preparation of ethanolic leaf extract of Canarium strictum (ELCS)

A soxhlet extractor was used to extract the shade dried *C.strictum* leaves (500g) using 90% pure ethanol at 60°C for 48h (Harborne, 1998). Under reduced pressure and temperature at 40°C, the crude extract was further concentrated using a rotary condenser and was further concentrated in an oven kept at 30°C overnight. (Selvam Rajkumar *et al*., 2022) Finally, 15g of a green solid was produced, which was stored at a temperature of 20°C was used for toxicological testing. Each day, the crude extract was freshly reconstituted in distilled water to the needed dosage level and administered orally.

HPTLC fingerprinting of scopoletin and ELCS

The high-performance thin layer chromatography (HPTLC) was used to identify the phytoconstituents present in the ELCS extract and further compared with scopoletin. Precoated Silica Gel 60 F 254 plates (10×10 cm) - stationary phase and Petroleum ether:ethyl acetate (1:1) - mobile phase was utilized. Using an automated Camag TLC applicator, samples and standards were placed on the plate in a 6mm wide band 15mm from the plate's bottom and 20 mm from its side. The plates were dried upon elution and further examined using a Camag UV chamber at 366nm and then the spots were removed (Tatke and Rajan, 2014).

Experimental studies

Swiss albino mice and Wistar rats were chosen respectively to conduct acute and sub-acute 90-day toxicity investigations (OECD, 2018). The experimental animals were procured from the National Institute of Mental Health and Neuroscience, Bangalore, India and transported to the Central Animal Facility in accordance with CPCSEA guidelines. In a sterilized polyacrylic cage (38×23×10cm), paddy husk bedding was used to house the experimental animals after they had been acclimatized for 21 days under 12:12 light:dark cycle, temperature maintained at 24±2°C and 30-70% relative humidity. Standard commercial rat/mice pellets (M/s. Hindustan Lever Ltd, Mumbai) were provided to all experimental animals and they had access to water at all times. The Institutional Animal Ethics Committee (1679/CPCSEA) at Anna University, Tiruchirappalli examined and approved the experimental study of animals and protocols vide AUROT/IAEC/NOV2013-0010.

Assessment of acute oral toxicity

Toxicity was evaluated in accordance with OECD recommendations (test number 423) (OECD, 2002) using female albino mice that were five to seven weeks old and weighed 50 to 70g. One dose of ELCS at 5, 50, 300 and 2000mg/kg was provided to overnight fasted female mice whereas the control group received distilled water and were observed twice a day for 14 days after treatment for signs of toxic effects, such as color change in the animal's skin, fur, eyes and mucous membranes; urination; defecation; autonomic activities; a change in posture, gait, seizures; aggression; and strange behavior. On days 1, 7 and 14, the weight of the rats was measured and recorded in this experiment. The animals were anaesthetized and then sacrificed by cervical dislocation for necropsy after the study. Kidney, heart, liver, lungs and spleen were collected by dissection.

Assessment of sub-chronic toxicity

The study was performed as per OECD test guidelines 408 (OECD, 2018) and applying good laboratory practices (Huntsinger, 2008). Four groups were randomly formed with both male and female Wistar rats (n=5 per group) weight ranged from 170 to 240g. Each group comprised 5 rats fed with standard pellet diet comprising 5% fat, 65% carbohydrate, 20.3% protein, 5% fiber, 3.7% salt mixture and 1% of vitamins. ELCS was administered orally to rats in treatment groups at doses of 300, 600 and 1200mg/kg/d, respectively for 90 days. The rats in the control groups received an equivalent dose of distilled water. During the course of experiment, the changes in the body weight of the experimental animals was recorded periodically at the end of every week. The experimental animals were monitored every day for mortality, changes in behavior and physical appearance as well as signs of disease in the animals. At the end of the treatment period, the rats were fasted overnight (12-16h) but allowed to drink water *ad libitum*. Then the rats were anaesthetized by intraperitoneal administration of urethane (1ml/100g body weight). Blood samples were collected from the abdominal aorta of sedated rats for hematological analysis and serum biochemistry in a EDTA (ethylene diamine-tetra acetic acid)-2K-coated and sterile dry blood tubes further centrifuged for 15 min at a speed of 3500×g to

isolate the serum for biochemical analysis. After euthanasia, the rats were sacrificed and internal organs were collected and weighed. The organs were processed and preserved histopathological investigation for necropsy (Hine, 1981).

Hematological and biochemical analysis

In order to do a hematological analysis, an automated analyzer was used (Sysmex SX100 fully automated). Previously, the tubes containing blood was centrifuged at 3000rpm for 15 min at the cooling temperature of 5°C to extract serum for assessing biochemical parameters. Measurements of hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC-is the ratio of the mean corpuscular hemoglobin (MCH) to the mean corpuscular cell volume (MCV), platelet (PLT), red blood cells (RBC), white blood cells (WBC) and platelet count were determined.

Biochemical analyzers (Chem 7 semi-automated) were used to evaluate the biochemical parameters i.e., Liver function-Total protein (TP), Albumin (ALB), bilirubin (BIL), alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST); Kidney function-Urea, Creatinine; lipid profile-low density lipoproteins (LDL), very low-density lipoproteins (VLDL), high density lipoprotein (HDL), total cholesterol (TC), triglycerides (TG) and glucose were among the clinical biochemistry parameters examined.

Weighing of organs

The thoracic and exterior surfaces as well as all internal organs in every single group, which was examined by performing a gross necropsy. Vital organs were surgically removed, washed using ice-cold saline solution, kept on absorbent papers, then weighed to determine total weight of the body (absolute organ weight in g) and thoroughly evaluated macroscopically for abnormalities. The relative organ weight (ROW) was estimated using this formula (Porwal *et al*., 2017):

Relative organ weight=Organ weight (g)-Rat's body weight (g) on sacrifice day×100.

Histopathology

Histopathological tests were carried out on the major organs as well as on the reproductive organs (testis and ovary) for the treated and control groups. To preserve the tissue specimens, ethanol was used to dehydrate them, toluene was used to clarify them and paraffin was used to enclose them. Microtome (Leica RM2235) was used to prepare sections with 5-m thickness, stained with hematoxylin and eosin (Hine, 1981), observed under the microscope, photographed and compared.

Analyses of statistical data

The obtained experimental data is presented as a mean and a standard deviation (SD) and evaluated for the statistical significance between the control and treatment groups by one-way ANOVA followed by Tukey's multiple comparison tests. Statistical analysis was performed using Graph Pad Prism version 6.0 for Windows. The differences between the treatment groups were considered statistically significant when the p-value<0.05.

RESULTS

HPTLC fingerprinting of scopoletin in ELCS

The presence of Scopoletin in ELCS was qualitatively confirmed and quantitatively determined. The Rf value of scopoletin was 0.16 and it was compared with ELCS Rf value of 0.18 (fig. 1). The mobile and stationary phase used offered a better resolution for the isolation and estimation of scopoletin in the crude extract.

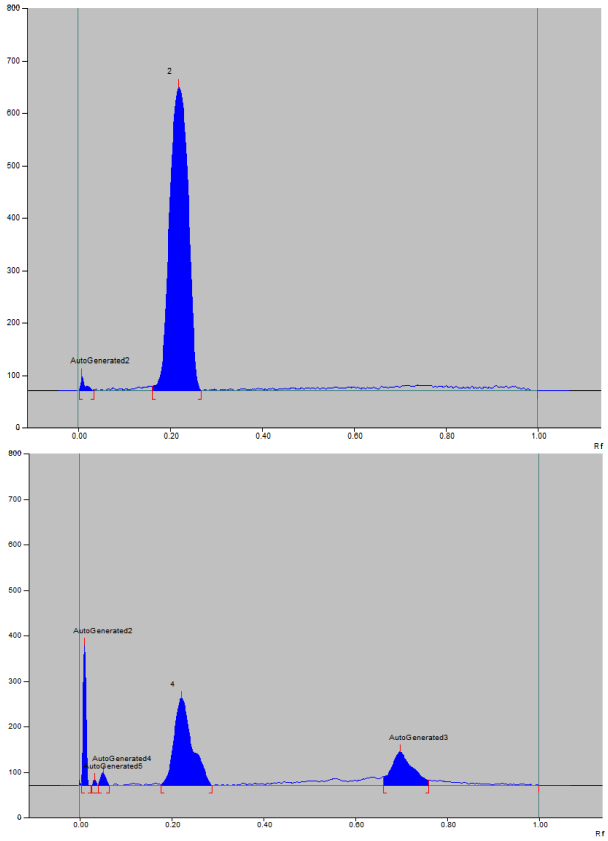

Fig. 1: (a) HPTLC chromatogram of standard scopoletin peak 1 (Rf = 0.16); mobile phase - petroleum ether:ethyl acetate (50:50v/v); (b) HPTLC chromatogram of ELCS; peaks 4 scopoletin (Rf = 0.18) components present in the extract.

Acute oral toxicity studies

The treated animals were monitored for 14 days following oral dosage in the sighting trial and no deaths were reported. During the course of the trial, the animals did not exhibit any symptoms of toxicity and poisoning. Normal look, grooming, posture, stride and behavior of

the animals were noted. However, neither an adverse effect nor a death was noted. Furthermore, no much changes in the body weight of the animals were recorded during the observation period. Since there were no abnormalities in gross pathology, a histological study was not performed.

Sub-chronic toxicity studies

The treated animals were monitored for 90 days following oral dosage, neither toxicity nor fatality was observed for the doses up to 1200mg/kg. During the course of the trial, the animals did not exhibit any symptoms of toxicity and poisoning. Normal look, grooming, posture, stride and behavior of the animals were noted. However, neither an adverse effect nor a death was noted. Furthermore, no much significant changes in the body weight of the animals were recorded during the observation period. The necropsy also identified no gross abnormal changes in the organs excised for investigation.

Recording of rat weight and clinical observations

Pattern of the body weights changes of animals belonging to the control and ELCS-treated groups are illustrated (figs. 2 & 3). Male and female rats in both the control and treatment groups gained similar amount of weight during the course of the experiment.

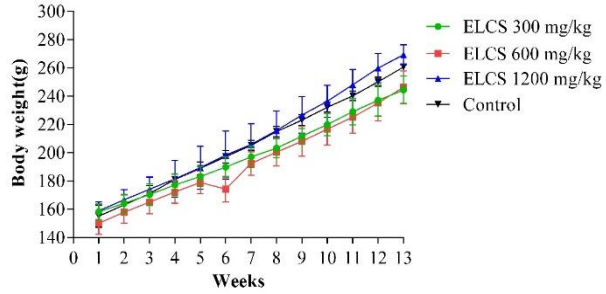

Fig. 2: Sub-chronic toxicity of ELCS-treated male rats. Each dot indicates the mean standard deviation (n=5).

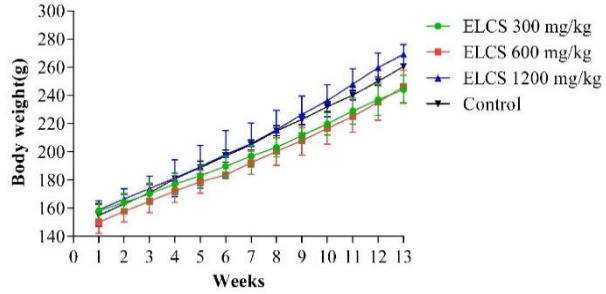

Fig. 3: Sub-chronic toxicity of ELCS-treated female rats. Each dot indicates the mean standard deviation (n=5).

Hematological analyses

ELCS (300, 600 and 1200mg/kg) sub-chronic oral treatment effects on rat hematological parameters (male and female) are mentioned (table 1). Both male and female rats showed significant changes in their hematological blood analyses. Changes in both groups of rats were not, however, contemporaneous or dose-related. Hemoglobin (HGB) and MCHC (mean corpuscular hemoglobin concentration), for example, showed a non-dose-dependent decrease ($p<0.05$) in female groups when administered at a dose of 1200mg/kg. Male rats-treated with 600mg/kg leaf extract showed a non-dose-dependent ($p<0.05$) increase in MCV when compared to the controls. Additionally, all of the assessed hematological tests were within normal ranges.

Clinical biochemical analysis

ELCS (300, 600 and 1200mg/kg) was administered sub-chronically orally to rats (females and males) and the results are shown (tables 2 & 3). Both male and female rats showed significant changes in their biochemical blood analyses. Changes in both groups of rats were not, however, contemporaneous or dose-related. An increase in HDL, LDL and total cholesterol at 1200mg/kg dosage was shown to be dose dependent ($p<0.05$) in male groups. A 600mg/kg dose of the extract revealed significant non-dose-dependent reductions in albumin and total protein levels in female rats, when compared with control. No significant changes were recorded in the case of AST and ALP in all the groups. Other biochemical parameters were found to be within acceptable ranges.

Organ weights in both absolute and relative terms

The weights of both male and female rats-treated with ELCS are mentioned (tables 4 & 5). The heart, liver, lungs and kidneys of both the control and treatment groups were found to be normal following the extract administration. When comparing the treatment groups to the control group, the absolute and relative organ weights recorded at necropsy did not differ significantly.

Histopathological analyses

Histopathological examinations of the heart, liver, kidneys and lungs were performed at the end of treatment. When comparing the ELCS-treated groups with the control groups microscopic examination revealed no abnormal pathological alterations in any of the organs (fig. 4). Treated groups didn't exhibit gross morphological changes.

DISCUSSION

There has been an increase in the utilization of plant-derived medicinal compounds in the treatment of major diseases in both developed and developing countries (Jillian and Guitelle, 2011). Life science and clinical researchers will benefit from examining and reporting on the safety and toxicity profiles of therapeutic plants. Comprehensive research is needed in order to forecast the risk of toxicity and offer scientific information for determining acceptable doses in humans for medicinal plant products (Mensah *et al*., 2019).

Silambarasan *et al*., 2015 reported the need for importance of documenting the traditional knowledge of forest dwelling people. *Canarium strictum* Roxb., was one of the plants which is recommended for further ethnopharmacological studies since these plants were proven with significant values.

Canarium strictum is one of the promising medicinal plants reported for the first time for the treatment of inflammation-related diseases widely used by the Lohit community. (Namsa *et al*., 2009).

Seethapathy *et al*., 2021 recently reviewed the Ethnopharmacology, biological activities and chemical compounds of *Canarium strictum* extensively. The resin of *Canarium strictum* Roxb. is used for rheumatism and asthma; the bark is used as a mosquito repellent. Triterpenoids and procyanidins are the major compounds in *C. strictum* resin and stem bark, respectively. The high content of triterpenoids might contribute to anti-inflammatory effects. This review confirms that the ELCS's safety profile has not been reported so far. Venkatachalapathi *et al.,* 2018 suggested to take-up the pharmacological and phytochemical studies to evaluate the ethnomedical potential of *Canarium strictum*. The essential oil composition and antibacterial activity of the resin of *Canarium strictum* Roxb. was investigated and reported. (Tahir *et al*., 2021)

As a result, the current study examined its toxicological profile in mice and rats over the course of 14 days using acute oral toxicity and sub-chronic oral toxicity, respectively. Toxicities and deaths were not observed in the 14-day study at the highest dose of 2000mg/kg of ELCS. There were no abnormalities in the animals' skin, fur, eyes, mucous membranes, respiratory system, autonomic nervous system, or behavioral activity during the 14-day experiment. In the acute toxicity research, female mice were found to be able to tolerate up to 2000mg/kg of ELCS extract. It was estimated that the LD_{50} of the extract via the oral route was more than 2000mg/kg. GSH, which was developed by the Organization for Economic Cooperation and Development, classifies chemicals as either class 5 drugs or non-toxic compounds (OECD, 2008).

Physiological alterations, such as hormonal changes, liver diseases and impaired absorption of proteins, amino acids and other nutrients, can be reflected in changes in body weight. Additionally, weight loss might affect the weight of internal organs (Thanabhorn *et al*., 2006). Early signals of drug and chemical toxicity can be assessed by changes made in the general behavior and body weight (Ezeja *et al*., 2014). There was no mortality or substantial behavioral abnormalities in the ELCS-treated female and male rats throughout sub-chronic poisoning. To our knowledge, there was no significant difference in mean body weight between the treatment and control groups throughout this investigation. Doses up to the level of 1,200mg/kg ELCS for the duration of 90 days were found to have no deleterious effect on the normal growth of rats (OECD, 2018).

It is important to evaluate risk based on blood parameters, as alterations in the hematological system play higher predictive value with respect to human toxicity (Olson *et al*., 2000). The hematopoietic system is regarded as the most sensitive target for hazardous substances and a key indicator for physiological and pathological conditions. The blood profile, on the other hand, is usually a good indicator of the body's response to trauma or stress (Gbolo *et al*., 2020). Compared to the control group, hematological parameters of neither male nor female-treated rats proved to be toxicologically significant. Significant changes in VCM (mean corpuscular volume) were recorded in male rats treated with ELCS (600mg/kg) in this study. Thus, these modifications were not sex- or dose-related. Toxicological importance was not assigned to these changes, as they were viewed as unrelated to treatment and not a result of exposure. Women administered with 1200mg/kg of the drug showed a decrease in HGB and MCHC (mean corpus hemoglobin concentration) levels compared to the female groups. As a result, this difference was not regarded as a harmful consequence because it did not occur in both female and male rats and dose-response association was unapparent.

Atherosclerosis, the first step toward coronary artery disease, is made more likely by hyperlipidemia. Hyperlipidemia and atherosclerosis are complicated diseases that are influenced by a number of interconnected genes (Wouters *et al*., 2005). In the recent years, cardiovascular pharmacology has focused on hypolipidemic (lipid-lowering) treatments, such as herbal medications and diets (Rouhi-Boroujeni *et al*., 2015). Lipid and lipoprotein (lipid-protein complexes) levels in the blood are reduced by many herbal medicines (Tajuddin *et al*., 2006). It was found that exposure to ELCS in the rats' food for 90 days increased the male rat's levels of total cholesterol, HDL and LDL, while this effect was not observed in the women's groups. That's why researchers did not consider this disparity harmful because it did not affect both genders.

Biotransformation of drugs occurs primarily in the liver. The biochemical characteristics of serum liver biomarker enzymes are commonly used to determine the liver's harmful effects (de Castro *et al*., 2017). ALT and AST serum transaminases and total protein levels are commonly used to detect liver impairment (Araújo *et al*., 2017). In the liver, there is a significant concentration of cytoplasmic enzyme ALT and an increase in the level of this enzyme indicates hepatocellular injury (Araújo *et al*., 2017). The liver, heart, skeletal muscle, kidney and brain all have substantial concentrations of AST in their cytoplasm and mitochondria (Arika *et al*., 2016; Ndrepepa and Kastrati, 2019).

Table 1: Hematological analysis of ELSC-treated male and female rats after 90 days

Gender	Parameter	ELCS treatment			
		Control	300 mg/kg	600 mg/kg	1200 mg/kg
Male	RBC	5.6±0.21	5.34±0.20	5.6±0.33	4.15±0.03****
	HGB (g/dL)	15±1.58	15.4±2.07	14.8±1.30	13.32±0.97
	HCT (%)	50.2±0.5	50.4±1.02	49.4±0.80	43±1.4****
	MCV	90.2±2.5	90.2±4.3	100.2±0.83	92.04±1.7
	MCH	29.8±1.9	30±1.8****	29.4±2.7****	28.98±0.56
	MCHC	33.6±2.0	33.6±1.14	32.2±1.9	32.28±1.17
	PLT	3.36±0.23	3.36±0.23	3.3±0.25	2.82±0.08**
	WBC	8800±543	8600±543	8840±320	6460±378****
Female	RBC	5.28±0.19	5.64±0.21	5.4±0.28	4.2±0.28 ****
	HGB (g/dL)	14.8±1.7	15±1.5	14.2±1.6	11.86±0.54*
	HCT (%)	59.2±0.70	49.6±0.63	49.7±0.79	43.66±1.0****
	MCV	87.6±2.79	90.2±2.5	89.6±1.4	89.2±1.64
	HCM	29.8±2.38	29.8±1.9	29.2±1.64	28.66±0.57
	MCHC	33.6±2.07	33.6±2.07	32.8±1.7	27.8±0.53***
	PLT	3.36±0.19	3.36±0.23	3.46±0.16	2.72±0.19***
	WBC	8700±758	8800±543	8940±260	6640±378****

n=5 each of males and females; Tukey's multiple comparisons and a one-way ANOVA was performed to calculate p value of significance; p=*-<0.5, **-<0.01, ***-<0.001 and ****-<0.0001; Cell count (RBC), hemoglobin (HGB), hematocrit (HCT), MCV, MCV, MCH, PLT and WBC count (WBC).

Table 2: Biochemical profile of ELCS-treated male rats after 90 days.

Parameter	ELCS treatment			
	Control	300 mg/kg	600 mg/kg	1200 mg/kg
AST (IU/L)	143.6±2.8	152±2.9**	148±2.9*	143.6±2.0
ALT (IU/L)	55.6±1.51	53±2.5	55.8±1.9	54.6±2.6
ALP (IU/L)	185.8±2.8	178.4±2.7**	188±1.7	185.6±1.5
Total protein (mg/dL)	66±3.3	70±3.6	67±1.5	66±2.5
Bilirubin (mg/dL)	0.52±0.01	0.53±0.02	0.53±0.01	0.54±0.02
Albumin (mg/dL)	41.35±1.1	42.2±0.66	40.89±1.9	42.35±1.8
Total cholesterol (mg/dL)	0.60±0.03	0.64±0.025	0.65±0.02	1.18±0.3**
Triglycerides (mg/dL)	0.75±0.01	0.73±0.05	0.74±0.01	0.67±0.4*
HDL cholesterol (mg/dL)	0.29±0.02	0.33±0.03	0.28±0.03	0.84±0.05***
LDL cholesterol (mg/dL)	0.29±0.02	0.29±0.01	0.30±0.01	0.74±0.08***
VLDL (mg/dL)	0.14±0.02	0.16±0.01	0.12±0.02	0.14±0.01
Glucose (mg/dL)	1.28±0.13	1.4±0.29	1.1±0.15	1.24±0.11
Urea (mg/dL)	0.34±0.022	0.34±0.12	0.31±0.02	0.34±0.01
Creatinine (mg/dL)	4.15±0.16	3.94±0.43	4.06±0.12	4.2±0.36

n=5 each of males and females; Tukey's multiple comparisons and a one-way ANOVA to calculate p value of significance; p=*-<0.5, **-<0.01, ***-<0.001 and ****-<0.0001.

Table 3: Biochemical profile of ELCS-treated female rats after 90 days.

Parameter	ELCS treatment			
	Control	300 mg/kg	600 mg/kg	1200 mg/kg
AST (IU/L)	142±2.9	148.2±2.16*	151±2.9***	144±1.5
ALT (IU/L)	54±2.1	50±2.77	55.6±1.6	54.2±2.3
ALP (IU/L)	184±2.4	186.2±2.77	177±2.1*	184.8±3.5
Total protein (mg/dL)	65.2±2.77	65.4±2.4	51±1.5***	64.6±1.9
Bilirubin (mg/dL)	0.52±0.03	0.49±0.02	0.55±0.01	0.52±0.03
Albumin (mg/dL)	41.8±0.52	42.24±0.6	32.2±1.4***	42.11±0.36
Total cholesterol (mg/dL)	0.63±0.01	0.64±0.01	0.64±0.02	0.65±0.03
Triglycerides (mg/dL)	0.71±0.05	0.73±0.02	0.72±0.12	0.68±0.05
HDL cholesterol (mg/dL)	0.27±0.03	0.26±0.12	0.29±0.06	0.27±0.02
LDL cholesterol (mg/dL)	0.31±0.03	0.31±0.02	0.28±0.02	0.32±0.01
VLDL (mg/dL)	0.14±0.02	0.13±0.01	0.12±0.03	0.14±0.03
Glucose (mg/dL)	1.16±0.20	1.06±0.20	1.1±0.15	0.96±0.3
Urea (mg/dL)	0.34±0.03	0.34±0.03	0.31±0.11	0.32±0.02
Creatinine (mg/dL)	4.18±0.04	4.0±0.19	4.0±0.12	3.96±0.37

n=5 each of males and females; Tukey's multiple comparisons and a one-way ANOVA to calculate p value of significance; p=*-<0.5, **-<0.01 and ***-<0.001.

Table 4: Body weight changes of ELCS-treated male rats after 90 days.

Organs	Control	*Canarium strictum* (mg/kg) B.W		
		300	600	1200
Heart	0.49±0.01	0.53±0.02	0.51±0.03	0.49±0.01
Lung	0.48±0.01	0.45±0.03	0.49±0.01	0.43±0.02*
Liver	3.67±0.10	3.84±0.26	3.76±0.24	3.88±0.32
Kidneys	0.74±0.02	0.76±0.03	0.75±0.02	0.78±0.03

The results are presented as the mean standard deviation (SD); n=5.

Table 5: Body weight changes of ELCS-treated female rats after 90 days.

Organs	Control	*Canarium strictum* (mg/kg) B.W		
		300	600	1200
Heart	0.50±0.02	0.49±0.02	0.50±0.05	0.51±0.01
Lung	0.47±0.01	0.44±0.04	0.49±0.01	0.44±0.02
Liver	3.60±0.09	3.73±0.34	3.68±0.24	3.79±0.34
Kidneys	0.74±0.02	0.75±0.02	0.75±0.03	0.78±0.04

The results are presented as the mean standard deviation (SD); n=5.

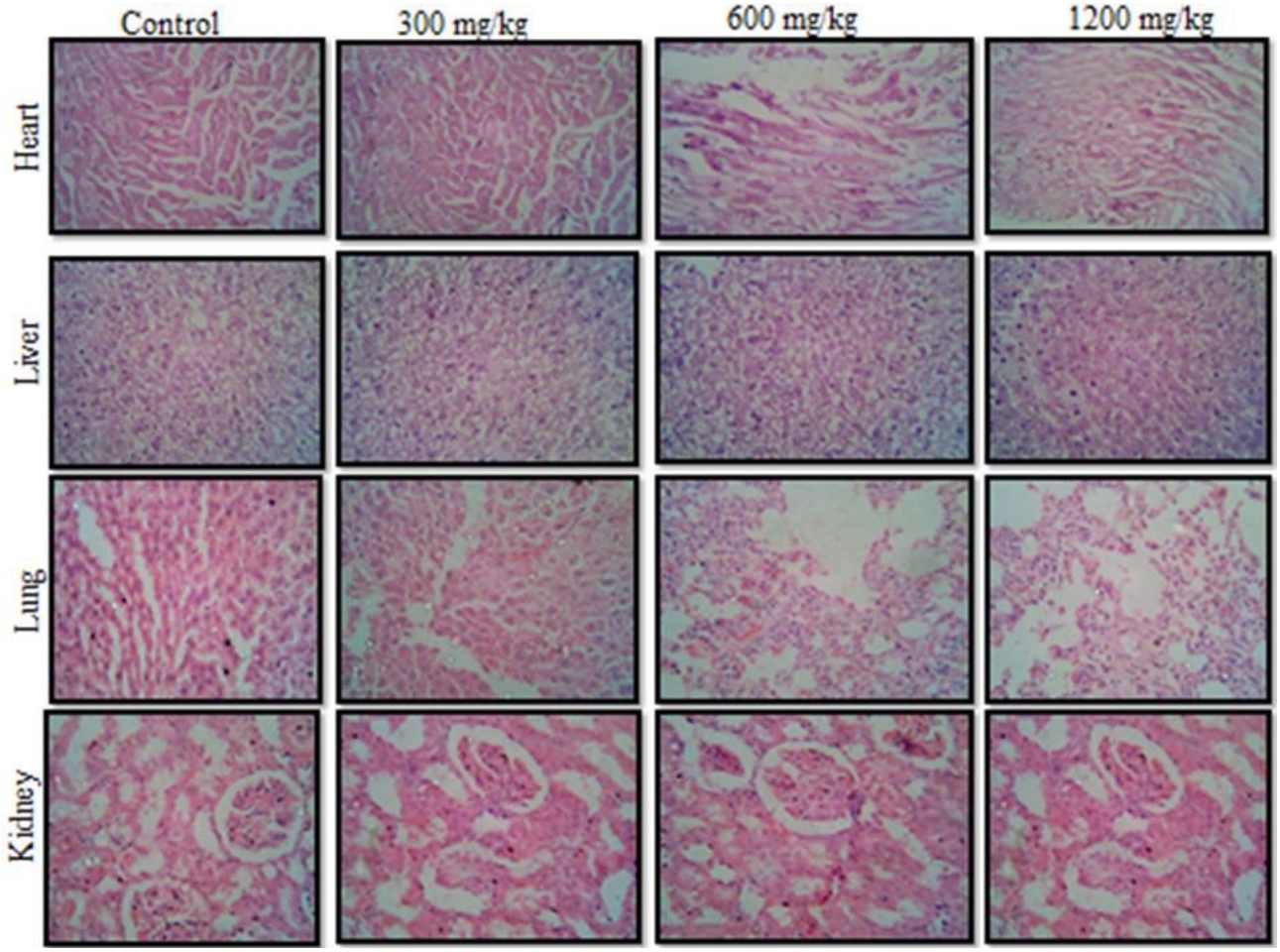

Fig. 4: Sections of vital organs of control and experimental groups treated with ELCS for 90 days. All of the treatment groups showed no meaningful change in their symptoms (x 40 magnification).

When comparing control and treated animals, there were no statistically significant variations in AST and ALT concentrations. Therefore, ELCS is safe for the liver. The livers of treated and untreated rats showed no abnormalities.

When a drug enters the circulatory system, it will reach the kidneys. Consequently, they are seen as a common target for toxicity. Renal diseases can be diagnosed using urine markers such as proteinuria, creatinine and urine urea nitrogen (UreN) (Wolf and Ziyadeh, 2007). No

significant changes were recorded in serum or creatinine levels in the rats treated by the leaf extract up to the medium and high doses compared to the control group and did not harm the kidneys, as reported earlier. There are many diseases associated with an increase in bilirubin levels, including primary biliary cirrhosis and hepatic cholestatic disease (Thapa and Walia, 2007). Protein status is measured using the serum total protein marker, which is an indicator of kidney and liver function. Infections or persistent inflammation in the liver may cause abnormal levels (Tatke and Rajan, 2014). Serum bilirubin levels in treated and untreated rats were not significantly different in this investigation. For the 600mg/kg-treated female rats, protein and albumin levels fall, a change not recorded in other treatment groups. According to previous reports on hematological parameters and liver function, the extract may not be hazardous to the erythropoietic system.

CONCLUSION

Though ELCS contains scopoletin that has been shown to be safe even at high concentrations the activity may be attributed to the synergistic effect of the compounds present in the leaves. The toxicity of the chemicals toward the targeted organs can be determined using relative organ weight measurements. If the tested substance has the potential to harm the organs, changes in their weight could be seen. A normal physical state of the organs was found throughout this study, with no signs of edema, atrophy or hyperplasia. A comparison of control rats-treated by ELCS revealed no difference in the relative organ weights of the two groups. Microscopic histopathological characteristics of the tissues were also found to be normal for all of the important organs.

ACKNOWLEDGMENTS

Thanks are extended to the Science and Engineering Research Board, Ministry of Science and Technology, India, for providing Dr. P. Selvamani with research grant under the Fast-Track Young Scientists Scheme [SERB Sanction Order No: SR/FT/LS-41/2010].

REFERENCES

Araújo MC, Barcellos NM, Vieira PM, Gouveia TM, Guerra MO, Peters VM and Saúde-Guimarães DA (2017). Acute and sub chronic toxicity study of aqueous extract from the leaves and branches of *Campomanesia velutina* (Cambess) O. Berg. *J. Ethnopharmacol.*, **201**(6): 17-25.

Arika WM, Nyamai DW, Osano KO, Ngugi MP and Njagi ENM (2016). Biochemical markers of *In vivo* hepatotoxicity. *J. Clin. Toxicol.*, **6**: 297.

De Castro CA, dos Santos Dias MM, da Silva KA, dos Reis SA, da Conceição LL, De Nadai Marcon L, de Sousa Moraes LF and do Carmo Gouveia Peluzio M (2017). Liver Biomarkers and Their Applications to Nutritional Interventions in Animal Studies. *In*: Patel VB, Preedy VR editors. Biomarkers in Liver Disease, Springer Netherlands, Dordrecht, pp. 129-152.

De Filipps RA and Krupnick GA (2018). The medicinal plants of Myanmar. *PhytoKeys*, **102**: 1-341.

Ezeja MI, Anaga AO and Asuzu IU (2014). Acute and sub-chronic toxicity profile of methanol leaf extract of *Gouania longipetala* in rats. *J. Ethnopharmacol.*, **151**(3): 1155-1164.

Fabricant DS and Farnsworth NR (2001). The value of plants used in traditional medicine for drug discovery. *Environ. Health Perspect.*, **109**(Suppl.1): 69-75.

Gbolo BZ, Ngbolua KN, Tshibangu DST, Memvanga PB, Tshilanda DD, Matondo A, Kilembe JT, Lebwaze BM, Nachtergael A, Mpiana PT and Duez P (2020). *In Vivo* evaluation and *in silico* prediction of the toxicity of Drepanoalpha hard capsules. bioRxiv. pp. 1-36.

Good laboratory practice training manual for the trainee: A tool for training and promoting good laboratory practice (GLP) concepts in disease endemic countries (2008), 2nd Edition, World Health Organization, Geneva. 1-238.

Halima Tahir, Nadeem Muhammad, Azeem Intisar, Muhammad Imran Din, Uzma Qaisar, Muhammad Abdul Qadir, Noor Ul Ain, Zeeshan Ahmad, Pakeeza Aziz, Muhammad Khurram Shahzad and Irshad Hussain (2021) Essential oil composition and antibacterial activity of *Canarium strictum* Roxb. resin. *Plant Biosystems.*, **155**(6): 1198 1202.

Harborne JB (1998). Textbook of *Phytochemical Methods*, A Guide to Modern Techniques of Plant Analysis, 5th Edition, Chapman and Hall Ltd., London. pp. 21-72.

Hine IF (1981). Block staining of mammalian tissues with hematoxylin and eosin. *Stain Technol.*, **56**(2): 119-123.

Hinge VK, Wagh AD, Paknikar SK and Bhattacharyya SC. Terpenoids-LXXI: Constituents of Indian black dammar resin. *Tetrahedron.*, **21**(11): 3197-3203.

Huntsinger DW (2008). OECD and USA GLP applications. *Ann. Ist. Super. Sanita*, **44**(4): 403-406.

Jillian CK and Guitelle BS (2011). The World Medicines Situation 2011 Good Governance for the Pharmaceutical Sector, World Health Organization, Geneva. 1-17.

Jin SE, Seo CS, Lee MY, Shin HK, Yang MJ and Ha H (2018). Sub-chronic toxicity of Gyejibokryeong-hwan in Sprague-Dawley rats. *J. Ethnopharmacol*., **224**(17): 441-450.

Meena D, Binaibabu N and Doss J (2012). Future prospects for the critically endangered medicinally important species, *Canarium strictum* Roxb. A review. *Int. J. Conserv. Sci.*, **3**(3): 231-237.

Mensah ML, Komlaga G, Forkuo AD, Firempong C, Anning AK and Dickson RA (2019). Toxicity and safety implications of herbal medicines used in Africa.

In: Builders PF editor. Herbal Medicine, IntechOpen, London. pp. 1-25.

Mogana R, Teng-Jin K and Wiart C (2013). Anti-inflammatory, Anticholinesterase and Antioxidant Potential of Scopoletin Isolated from *Canarium patentinervium* Miq. (Burseraceae Kunth), *Evid. Based Complementary Altern. Med.*, pp.1-7.

Muthuswamy R (2013). Anti-inflammatory activity of essential oil of *Canarium strictum* Roxb. *Iran. J. Pharm. Sci.*, **9**(2): 13-21.

Nadkarni KM (1989). Indian Materia Medica, Popular Prakashan, Bombay. p. 254.

Namsa ND, Tag H, Mandal M, Kalita P and Das AK (2009). An ethnobotanical study of traditional anti-inflammatory plants used by the Lohit community of Arunachal Pradesh, India. *J. Ethnopharmacol.*, **125**(2): 234-45.

Ndrepepa G and Kastrati A (2019). Alanine aminotransferase a marker of cardiovascular risk at high and low activity levels. *J. Lab. Precis. Med.*, **4**: 29.

OECD (2002). Test No. 423: Acute oral toxicity - acute toxic class method, OECD guidelines for the testing of chemicals, Section 4, OECD Publishing, Paris.

OECD (2008). Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

OECD (2018). Test No. 408: Repeated dose 90-day oral toxicity study in rodents, OECD guidelines for the testing of chemicals, Section 4, OECD Publishing, Paris.

Oliveira MJA, van Deventer HE, Bachmann LM, Warnick GR, Nakajima K, Nakamura M, Sakurabayashi I, Kimberly MM, Shamburek RD, Korzun WJ, Myers GL, GregMiller, W and Remaley AT (2013). Evaluation of four different equations for calculating LDL-C with eight different direct HDL-C assays. *Clin. Chim. Acta.*, **423**: 135-140.

Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Deun KV, Smith P, Berger B and Heller A (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul. Toxicol. Pharmacol.*, **32**(1): 56-67.

Porwal M, Khan NA, Maheshwari KK. (2017) Evaluation of acute and subacute oral toxicity induced by ethanolic extract of *Marsdenia tenacissima* leaves in experimental rats. *Scientia Pharmaceutica.*, **85**(3): 29.

Ribeiro RV, Mariano DB, Arunachalam K, Soares IM, Aguiar RW de S, Ascencio SD, Marlon R, Colodel EM and Martins DT de O (2019). Chemical characterization and toxicological assessment of hydroethanolic extract of *Mandevilla velame xylopodium*. *Rev. Bras. Farmacogn.*, **29**(5): 605-612.

Rouhi-Boroujeni H, Rouhi-Boroujeni H, Heidarian E, Mohammadizadeh F and Rafieian-Kopaei M (2015). Herbs with anti-lipid effects and their interactions with statins as a chemical anti- hyperlipidemia group drugs: A systematic review. ARYA *Atheroscler.* **11**(4): 244-51.

Sarker MR (2014) Therapeutic efficacy and safety of traditional Ayurvedic Medicines: Demands for extensive research and publications. *J. Homeop. Ayurv. Med.*, **3**(3): e113.

Seethapathy GS, Wold CW, Ravikumar K, de Boer HJ and Wangensteen H (2021) Ethnopharmacology, biological activities and chemical compounds of *Canarium strictum*: An important resin-yielding medicinal tree in India. *Fitoterapia.* **152**(5): 104920.

Selvam Rajkumar, Subbiah Latha, Thangavelu Prabha, Mathew Joseph Arputha Bibiana and Palanisamy Selvamani (2022) Scopoletin, a biomarker isolated from *canarium strictum* and its ameliorating potential on scopolamine-induced cognitive and behavioral impairment in rats. *Neuro Quantology.*, **20**(6): 6529-6540.

Silambarasan R and Ayyanar M (2015) An ethnobotanical study of medicinal plants in Palamalai region of Eastern Ghats, India. *J. Ethnopharmacol.*, **172**(14): 162-78.

Suruse PB, Duragkar N, Shivhare U and Bodele S (2010). Study of antimicrobial activity of *Canarium strictum* gum resin. *Res. J. Pharmacogn. Phytochem.*, **2**(6): 437-439.

Tahir H, Muhammad N, Intisar A, Din MI, Qaisar U, Qadir MA, Ain NU, Ahmad Z, Aziz P, Shahzad MK and Hussain I (2021). Essential oil composition and antibacterial activity of *Canarium strictum* Roxb. resin. *Plant Biosys.*, **155**(6): 1198-1202.

Tajuddin, Nasiruddin M and Ahmad N (2006). Effect of an unani formulation on lipid profile in rat. *Indian J. Pharmacol.*, **38**(1): 56-57.

Tatke P and Rajan M (2014). Comparison of conventional and novel extraction techniques for the extraction of scopoletin from *Convolvulus pluricaulis*. *Indian J. Pharm. Educ. Res.*, **48**(1): 27-31.

Thanabhorn S, Jaijoy K, Thamaree S, Ingkaninan K and Panthong A (2006). Acute and subacute toxicity study of the ethanol extract from *Lonicera japonica* Thunb. *J. Ethnopharmacol.*, **107**(3): 370-373.

Thapa BR and Walia A (2007). Liver function tests and their interpretation. *Indian J. Pediatr.*, **74**(7): 663-671.

Venkatachalapathi A, Sangeeth T, Ali MA, Tamilselvi SS, Paulsamy S, Al-Hemaidc FMA. Ethnomedicinal assessment of Irula tribes of Walayar valley of Southern Western Ghats, India. (2018) *Saudi J. Biol. Sci.*, **25**(4): 760-775.

WHO (2019). WHO Global report on traditional and complementary medicine. World Health Organization, Geneva. Licence: CC BY-NC-SA 3.0 IGO.

Wolf G and Ziyadeh FN (2007). Cellular and molecular mechanisms of proteinuria in diabetic nephropathy. *Nephron Physiol*., **106**(2): 26-31.

Wouters K, Shiri-Sverdlov R, van Gorp PJ, van Bilsen M and Hofker MH (2005). Understanding hyperlipidemia and atherosclerosis: Lessons from genetically modified apoe and ldlr mice. *Clin. Chem. Lab. Med.*, **43**(5): 470-479.

Zhu M, Lew KT and Leung PL (2002). Protective effect of a plant formula on ethanol-induced gastric lesions in rats. *Phytother. Res.*, **16**(3): 276-280.