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RESEARCH ARTICLE

Short and long-term safety of leaf extracts of *Lagerstroemia speciosa* (L.) Pers. in maintaining blood glucose and lipid levels as revealed by phytochemical and pharmacological analysis

Sajan JS[1], Karuna Surendran[2], Suja SR[1] & Padmesh P Pillai[2*]

[1]Biotechnology & Bioinformatics Division, Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Palode, Thiruvananthapuram 695 562, Kerala, India
[2]Department of Genomic Science, Central University of Kerala, Kasaragod 671 325, Kerala, India

***Correspondence email -** padmeshpillai@cukerala.ac.in

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Abstract

Lagerstroemia speciosa is a woody tree with numerous therapeutical properties that improve metabolic defects caused by elevated glucose and lipid levels. We have investigated the safety and efficacy potentials of *L. speciosa* leaf extract (LSLE) by conducting toxicity studies comprising acute short-term studies of 28 days and long-term studies of 90 days duration in rodent models of both sexes. The acute toxicity studies revealed that the LD_{50} value exceeded 3000 mg/kg of body weight. For the sub-chronic toxicity assessment, Wistar rats were administered LSLE concentrations orally every day for 28 days, with a control group receiving 0.5% Tween 80. A notable reduction in body weight gain, food intake, glucose and pancreatic amylase levels was recorded in the LSLE-treated group. These crucial findings were validated in a chronic toxicity study involving Wistar rats, which were orally administered LSLE daily for 90 days. The percentage of food intake was observed to decrease progressively. Nevertheless, no remarkable alterations were noted in the hematological parameters, histological analyses, relative organ weights, or other biochemical parameters, except for glucose and pancreatic amylase. The overall findings indicate that long-term administration of LSLE is non-toxic and very effective in maintaining the percentage of body weight gain through multiple mechanisms.

Keywords: acute toxicity; banaba; corosolic acid; efficacy; herbal medicine; HPLC; LC-MS

Introduction

Traditional medicine and herbal products have been widely used throughout the ages worldwide due to their accessibility, affordability, lower risk and relatively fewer side effects. It is estimated that the majority of the population in developing countries meet their primary healthcare needs with traditional medicines. However, the acceptance of these products has been limited due to the lack of precise chemical characterization, sufficient toxicity data and standardized dosing regimens, all of which are crucial for assessing their efficacy and safety (1).

Lagerstroemia speciosa, a woody shrub or small tree from the Lythraceae family, is valued for its medicinal properties and is widely referred to as banaba or Pride of India (2). Initially native to the tropical regions of Southeast Asia and the Indo-Malayan region, it has since expanded to temperate and tropical regions worldwide (3). They are predominantly admired for their visual appeal and decorative value, featuring prolonged, beautiful, vibrant and stunning flowers in various colors, facilitating numerous crossbreeds both within and between species. Through natural and artificial hybridization, flowers with diverse hues were obtained, generating high demand in both national and international markets. Apart from its aesthetic properties, *L. speciosa* is widely used traditionally in various forms to treat many diseases, such as cardiovascular diseases, high blood pressure, inflammation, hypertension, diabetes and kidney-related ailments (4 - 8). The phytochemical analysis identified an array of bioactive compounds such as lagerstroemin, reginin A and flosin B acetal, ellagic acid, sterol, alkaloids, tannins, tannic acid along with pentacyclic triterpenes such as oleonic acid, asiatic acid, arjunolic acid, corosolic acid and maslinic acid from different extracts of *L. speciosa* (9 - 14). These phytochemicals attribute to antidiabetic, antiobesity, antioxidative, antiseptic and anticancer properties of the species (4, 5, 15 - 17). Recently, the ethanolic banaba leaf extract (EBLE) has been reported to have anticancer properties against HepG2 cells. The HPTLC analysis validated the presence of phytochemicals like corosolic acid, gallic acid and berberine in EBLE (18).

Medicinal plants, though generally considered safe, are not free from the risk of toxicity (19). Therefore, many herbal extracts were reported as neurotoxic, nephrotoxic,

hepatotoxic and occasionally fatal leading to death (20-27). To develop Improved Traditional Medicines (ITMs) that are safe, affordable and effective. Accessible for drug target identification, it is proposed that pre-clinical testing of botanicals should start with *in vivo* studies using suitable animal models to confirm their ethnopharmacological applications (28). Herbal medicines and dietary supplements are often seen as safe. Still, since they are not regulated under the same criteria as drugs by the US-FDA, there may be insufficient documentation regarding their safety. Preclinical toxicological evaluations conducted in various modes, following the guidelines of the Organization for Economic Cooperation and Development (OECD), are essential for determining the safety profiles of drugs derived from herbs (29).

Despite the extensive knowledge of this plant's biological activities and documented usage, information on its presumed toxicity remains limited (30, 31). Therefore, is the current investigation aims to assess the short-term and long-term toxicity of *L. speciosa* leaf extract (LSLE), if any, by following the suggested OECD guidelines to assess the safety or dose-dependent toxicity in rodents.

Material and Methods

2.1 Plant Sample

L. speciosa leaves were collected from the JNTBGRI campus during the summer season. Dr. A.G. Pandurangan of JNTBGRI identified the plant sample and the specimen was archived in the Institute's herbarium under the reference number TBGT-65821.

The leaves were dried in the air and then powdered (500 g) and extracted using a Soxhlet apparatus with 2.5 L of methanol for 8 h. After extraction, the solvent was fully evaporated using a rotary evaporator under controlled pressure (Heidolph, Germany) and the yield of extract (w/w) was recorded (49 g).

2.2 Animals

The study employed adult Wistar rats, aged 90 days, with females weighing 140 - 200 g and males weighing 150 - 220 g, as well as albino mice, aged 60 days, with females weighing 25 - 35 g and males weighing 35 - 45 g. The animals were given ad libitum access to a commercial diet (Lipton India Ltd, Mumbai, India) and boiled water. They were housed at a temperature control of (25 ± 1 °C) with 12 h photoperiod in the vivarium at the JNTBGRI campus. All animal procedures were conducted in strict compliance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health and also with the standards established by the Animal Welfare Act. The study was conducted with the approval of the Institute's Ethics Committee (Protocol No. B/03/01/2013/06). All toxicological studies were performed according to OECD Guidelines 1995, followed by updated OECD recommendations 2001 (OECD 407 for sub-chronic and 408 for chronic studies) with the approval of the Institute Ethics Committee.

2.3 Acute toxicity study in Wistar rats

The method was carried out in line with the OECD test guidelines, with minor alterations (32). Albino mice were divided into 7 groups, each comprising 12 animals, with 6 males and 6 females. Six groups received oral treatments with varying doses of LSLE (50, 100, 200, 400, 800, 1600 and 3000 mg/kg body weight), solvated in a 0.5% Tween 80 solution. The group that received 0.5% Tween 80 constituted the negative control. Each mouse was administered a volume of approximately 0.5 mL. The animals were provided unrestricted access to food and water. The general behaviour and the number of survivors were monitored at intervals of 5, 10, 15, 20 and 30 min, as well as at 1, 2, 3, 4, 5, 10 and 24 h and then daily until day 14. During this period, adverse toxicological effects were evaluated, including diarrhoea, drooling, respiration, locomotion, altered muscle tone, writing, hyper-excitability and mortality. The animals' body weights were recorded throughout the entire experimental period. All the mice were sacrificed at the completion of the experiment. The internal organs were removed and evaluated for bare morphological abnormalities.

2.4 Sub-chronic toxicity studies in Wistar rats

The procedure was carried out according to the OECD test protocols, with some minor modifications . Three-month-old Wistar rats were grouped into 4, each consisting of 12 animals (6 males and 6 females). Three groups received oral administration of varying doses of LSLE (100, 200, 400 mg/kg body weight) resuspended in 0.5% Tween 80 (vehicle) over 4 weeks. Approximately 1.00 mL was administered to each animal. The animals were regularly monitored for signs of toxicity and mortality throughout the experiment. Body weight and behaviour were assessed and recorded weekly for each rat. Food and water intake were recorded thrice a week. On the final day of the experiment, the animals were kept unfed for 12 h before being sacrificed by carbon dioxide inhalation. Blood was secured immediately into tubes for the analysis of hematological parameters and to obtain serum as well. The vital organs like the heart, kidney, liver, lungs and spleen were weighed and fixed in 10% formalin for histopathological examination.

2.5 Chronic toxicity studies in Wistar rats

The procedure was conducted in accordance with the OECD test guidelines, with some minor modifications . Three-month-old Wistar rats were arbitrarily grouped into 4 groups, each containing 12 animals, comprising 6 males and 6 females. They received different doses orally daily under the same conditions as previously described, up to 90 days. All animals had unrestricted reach to food and water throughout the treatment period and were closely monitored daily, with their weight documented weekly. On the 90th day, the animals were fasted and then euthanized as previously described. Subsequently, blood samples were retrieved for various analysis. The organs were meticulously dissected, removed and weighed for histopathological examination. ALP, AST, ALT, bilirubin, protein, cholesterol, pancreatic amylase, triglycerides, HDL, creatinine, albumin and urea were evaluated using standard analytical kits from Crest Biosystems, Goa, India. LDL and VLDL were calculated by Friedwald's formula (33).

Hematological analyses were conducted using an automatic counter (Mindray-BC 2800-vet, China) to assess

various parameters, including haemoglobin, RBCs, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count and total leucocyte count. Differential WBC counts were also assessed, comprising neutrophils, basophils, eosinophils, lymphocytes and monocytes.

2.6 Organ weight and histopathological analyses

The vital body organs such as the heart, kidney, liver, lungs and spleen were examined macroscopically in all the animals. The position, size, shape and color of these internal organs were examined for any visible symptoms of macroscopic abnormalities and then weighed. Subsequently, slides were prepared for microscopic analysis using hematoxylin and eosin.

2.7 Qualitative and quantitative analysis of LSLE

LCMS was performed on an Agilent 1260 infinity with a 6120 Quadrupole system using a positive electro spray Atomic pressure ionization (ES-API) source in the scan mode. The sample was dissolved in methanol and filtered through a 0.2 µm nylon filter. The chromatography-based segregation was conducted on an Eclipse plus C-18 column 5 µm particle size, 250 X 4.6 mm (Agilent technologies). The column temperature was maintained at 30 °C and the injection volume was set at 10 µL. Methanol and formic acid (95 : 5) at a flow rate of 1 mL/min constituted the mobile phase.

HPLC analysis of the constituents such as corosolic acid, gallic acid and oleanolic acid was performed by HPLC with an Agilent 1260 series infinity system (Agilent, Waldbronn, Germany) using a C-18 column (3.5 µm, 4.5 mm × 100 mm) at 26 °C, with a flow rate of 1.0 mL/min. The mobile phase consisting of 1% Phosphoric acid : Methanol (80 : 20) operated at 280 nm was used for the analysis of gallic acid and Acetonitrile : 1% Phosphoric acid in water (75 : 25) operated at 210 nm was used for the analysis of corosolic acid and oleanolic acid. The constituents of the extract were quantified by comparison with their respective authentic standards (Sigma-Aldrich, USA).

Statistical analysis

The results are presented as the mean ± standard deviation (SD). Statistical analyses were conducted using two-way analysis of variance (ANOVA) using GraphPad Prism software, version 5.04, followed by Bonferroni multiple comparison tests.

Results

3.1 Acute toxicity study in mice

The different doses of LSLE (50, 100, 200, 400, 800, 1600, 3000 mg/kg body weight) showed no indication of toxicity and the body conditions such as behaviour, breathing, nervous system responses, cutaneous effects and gastrointestinal responses were almost normal. No body weight increase was observed in male or female mice; however, a decrease in food and water consumption was noted (data not shown). LSLE up to the dosage of 3000 mg/kg showed no adverse effects during the 15-day observation phase, suggesting that the median lethal dose (LD50) for both male and female mice is more significant than 3000 mg/kg. Additionally, gross examinations of the internal organs of the treated mice exhibited no pathological abnormalities when compared to the control group.

3.2 Sub chronic toxicity study in Wistar rats

In the sub-chronic toxicity studies, LSLE at various doses, given orally, did not result in any mortality or toxicity in Wistar rats. Significant variations in general behaviour or any other physiological abnormalities were not detected throughout the study period. Variations in body weight among all groups were monitored for 28 days and summarized in (Table 1). The intake of food and water was recorded weekly throughout the duration of the experiment phase (Table 1**)**. All groups exhibited weight gain when compared to their initial weight. Additionally, compared to the control group, the percentage of body weight gain in treated both male and female groups was inversely related to increasing the dosage of LSLE (Table 2).

3.3 Hematological parameters

The hematological profile of both the test and control groups

Table 1. Impact of sub-chronic oral dosing of LSLE (mg/kg/day) on body weight, food intake and water consumption in Wistar rats

Week (w)	Normal (Male)	Normal (Female)	100 (Male)	100 (Female)	200(Male)	200 (Female)	400 (male)	400 (Female)
Body weight (g)								
W0	145.33 ± 1.36	134.33 ± 0.51	148 ± 1.54	138.66 ± 1.03	149 ± 1.78	140 ± 0.89	155.33 ± 1.36	149.66 ± 1.36
W1	151 ± 2.68	141 ± 0.89	155 ± 0.89	144.66 ± 1.86	155 ± 0.89	146.33 ± 1.03	159.3 ± 0.54	155.66 ± 0.51
W2	157 ± 1.54	145.33 ± 1.36	162 ± 0.89	152 ± 2.36	161.33 ± 2.87	149.83 ± 1.12	164.33 ± 0.51	158.73 ± 0.98
W3	161.66 ± 2.58	152.33 ± 1.03	166.66 ± 1.03	157.33 ± 2.87	167.33 ± 2.87	155.16 ± 0.93	168.33 ± 1.36	163.7 ± 1
W4	166.66 ± 2.25	157.1 ± 0.76	172 ± 0.89	164.66 ± 1.86	171.73 ± 2.55	159.73 ± 1.38	172.66 ± 1.03	167.4 ± 0.86
Food intake (gm/day/rat)								
W1	20.53 ± 0.54	19.84 ± 0.66	21.57 ± 0.48	19.84 ± 0.25	21.92 ± 0.13	20.38 ± 0.64	22.16 ± 0.81	21.92 ± 0.57
W2	19.04 ± 0.45	19.05 ± 0.49	20.68 ± 0.48	18.31 ± 0.37	19.38 ± 0.52	18.93 ± 0.41	20.12 ± 0.93	18.77 ± 0.2
W3	18.73 ± 0.25	17.78 ± 0.51	18.74 ± 0.76	18.78 ± 0.6	18.66 ± 0.46	18.28 ± 0.41	18.91 ± 0.66	18.11 ± 0.46
W4	18 ± 0.09	18.14 ± 0.39	18.03 ± 0.35	18.28 ± 0.1	18.13 ± 0.28	18.13 ± 0.19	18.32 ± 0.1	17.88 ± 0.24
Water intake (mL/day/rat)								
W1	22.67 ± 0.35	21.97 ± 0.52	22.76 ± 0.54	21.16 ± 0.68	22.3 ± 0.28	21.36 ± 0.24	22.52 ± 0.2	21.82 ± 0.49
W2	22.26 ± 0.13	21.5 ± 0.35	22 ± 0.76	20.72 ± 0.33	21.74 ± 0.49	20.75 ± 0.54	21.96 ± 0.45	20.77 ± 0.57
W3	21.48 ± 0.45	20.77 ± 0.52	21.37 ± 0.3	20.17 ± 0.36	20.74 ± 0.47	20.25 ± 0.21	21.05 ± 0.73	19.99 ± 0.36
W4	19.44 ± 0.61	19.47 ± 0.45	20.02 ± 0.49	18.91 ± 0.32	19.53 ± 0.43	18.29 ± 0.56	18.33 ± 0.57	18.6 ± 0.56

Data are expressed as mean ± SD (n = 6). Two-way ANOVA followed by Bonferroni multiple comparison tests. W0 = Initial week, W1 = first week, W2 = second week, W3 = third week, W4 = fourth week.

Table 2. Percentage increase in body weight of Wistar rats following 28 days of treatment with LSLE

Groups (28 Days)	Weight gain (%)
N - Male	14.67 ± 2.25
N - Female	16.94 ± 0.76
100 - Male	16.21 ± 0.89
100 - Female	18.75 ± 1.86
200 - Male	15.25 ± 2.55
200 - Female	14.09 ± 1.38
400 - Male	11.15 ± 1.03
400 - Female	11.84 ± 0.86

Data are represented as the mean ± SD (n = 6). Statistical significance was tested using two-way ANOVA ($P < 0.05$).

are represented in Table 3. The results indicate that LSLE did not alter the hematological parameters considerably, including hemoglobin levels, WBC counts, total erythrocyte count, erythrocyte indices (MCV, MCH, MCHC), or the total red blood cell and platelet counts. All hematological parameters persisted within the normal physiological range, indicating no significant differences between the treated male and female rats in comparison with the control groups, throughout the experimental period.

3.4 Biochemical analysis

LSLE administration did not result in any notable changes in the biochemical profiles such as ALP, AST, ALT, albumin, bilirubin, globulin, protein, triglycerides, creatinine, HDL, LDL and VLDL, relative to the standard control groups except glucose and pancreatic amylase. Correlated with the control groups, the levels of glucose and pancreatic amylase were in descending order as with the increase in dosage, both in the treated male and female groups (Table 4).

3.5 Relative organ weight and histopathology

The relative organ weights of the LSLE-treated groups did not show any significant differences. The cellular morphology, tissue integrity and nuclear characteristics of organs such as the heart, kidney, liver, lungs and spleen in the treated groups were similar to those in the control groups and revealed standard architecture.

3.6 Chronic toxicity studies in Wistar rats

Interestingly, there is no indication of toxicity or any noticeable changes observed in animal behaviour, breathing pattern, sensory nervous system responses, gastrointestinal effects and cutaneous effects in treated male and female Wistar rats during the long-term experimental period of 90 days. The percentage of food and water intake during the investigation period was

Table 3. Impact of sub-chronic oral dosing of LSLE (mg/kg/day) on the hematological parameters in Wistar rats

Parameter	Normal (Male)	Normal (Female)	100 (Male)	100 (Female)	200 (Male)	200 (Female)	400 (male)	400 (Female)
RBC ($X10^6$ /µL)	7.1±0.08	6.36±0.36	7.33±0.28	6.93±0.26	7.13±0.06	6.88±0.15	7.14±0.09	6.53±0.32
HG (g/dL)	13.36±0.22	13.7±0.33	13.8±0.49	13.37±0.49	13.58±0.3	13.43±0.21	13.89±0.13	14.04±0.44
MCV(fL)	55±0.09	60±0.02	58±0.01	61±0.09	50±0.04	63±0.05	60±0.03	60±0.08
MCH (pg)	18.7±0.058	20.8±0.16	18.7±0.01	19.3±0.14	19±0.05	194±0.07	194±0.03	21.1±0.13
MCHC (g/dL)	31.36±1.05	31.43±0.4	31.96±0.13	31.6±0.55	31.72±0.72	30.32±0.32	31.87±0.29	30.76±0.49
WBC($X10^3$ /µL)	3.38±0.15	2.56±0.18	2.97±0.13	3.23±0.4	3.5±0.32	2.96±0.22	3.16±0.22	2.91±0.14
Neautrophyl (%)	17.83±1.35	15.2±1.17	16.69±1.4	16.83±1.53	16.05±0.97	16.8±0.39	16.01±0.95	15.66±0.36
Lymphocytes (%)	76±1.78	71.5±0.44	75.36±1.16	73.36±1.35	76.5±2.48	73.09±0.88	74.36±2.74	73.41±1.45
Monocytes (%)	4.84±0.28	4.85±0.43	5±0.23	4.32±0.21	4.91±0.42	4.81±0.31	4.99±0.28	5.17±0.13
Eosinophyl(%)	1.77±0.19	1.35±0.36	1.82±0.13	1.44±0.29	1.76±0.12	1.54±0.09	1.98±0.25	1.47±0.38

Data represented as mean ± SD (n = 6). Statistical significance was tested using one-way ANOVA (P<0.05) followed by Bonferroni multiple comparison tests. RBC = red blood cells, HG = hemoglobin, MCV = mean corpuscular volume, MCH = mean cell hemoglobin, MCHC = mean corpuscular hemoglobin concentration, WBC = White blood cells.

Table 4. Impact of sub-chronic oral dosing of LSLE (mg/kg/day) on the biochemical parameters in Wistar rats

Parameter	Normal (Male)	Normal (Female)	100 (Male)	100 (Female)	200 (Male)	200 (Female)	400 (male)	400 (Female)
Albumin	5.09 ± 0.17	4.92 ± 0.18	4.96 ± 0.27	4.94 ± 0.19	5.08 ± 0.17	4.88 ± 0.03	5.09 ± 0.32	5.04 ± 0.20
ALP	3.36 ± 0.22	2.99 ± 0.03	2.41 ± 0.09	2.13 ± 0.05	3.27 ± 0.10	2.85 ± 0.11	3.04 ± 0.19	3.06 ± 0.16
Bilirubin	1.80 ± 0.02	1.72 ± 0.02	1.86 ± 0.03	1.86 ± 0.03	1.90 ± 0.02	1.87 ± 0.02	1.89 ± 0.02	1.87 ± 0.03
Creatine	0.38 ± 0.03	0.31 ± 0.02	0.43 ± 0.02	0.34 ± 0.02	0.46 ± 0.03	0.42 ± 0.02	0.47 ± 0.03	0.39 ± 0.01
Globulin	2.3 ± 0.37	2.54 ± 0.17	2.44 ± 0.32	2.35 ± 0.15	2.21 ± 0.29	2.23 ± 0.06	2.27 ± 0.14	2.31 ± 0.18
Glucose	121.66 ± 4.22	107.66 ± 3.61	118 ± 4.09	110 ± 2.68	118.66 ± 2.87	107.6 ± 2.78	116 ± 4.09	103.6 ± 7.3**
Pancreatic amylase	2.25 ± 0.15	2.23 ± 0.28	2.13 ± 0.09	2.14 ± 0.18	2.32 ± 0.17	2.32 ± 0.17	2.01 ± 0.19	1.93 ± 0.11*
Protein	7.4 ± 0.23	7.47 ± 0.02	7.4 ± 0.08	7.29 ± 0.04	7.29 ± 0.2	7.08 ± 0.03	7.37 ± 0.18	7.35 ± 0.08
AST	86.33 ± 4.22	80.66 ± 2.73	83 ± 2.68	76.33 ± 6.08	87.66 ± 2.06	78.66 ± 5.08	88 ± 2.36	80.66 ± 1.86
ALT	28.33 ± 1.86	23 ± 0.89	28.33 ± 2.87	24.66 ± 1.36	28 ± 2.36	26 ± 1.78	29.33 ± 2.25	26 ± 2.36
Urea	40.66 ± 2.06	37 ± 2.36	42.33 ± 1.86	39.33 ± 1.86	46 ± 0.89	46.36 ± 1.05	44.33 ± 1.36	42 ± 0.89
Triglycerides	129.89 ± 2.52	119.63 ± 2.13	119.74 ± 9.81	123.24 ± 2.33	145.5 ± 2.4	138.7 ± 1.401	134.96 ± 4.33	119 ± 11.41
HDL	36.66 ± 1.36	32 ± 0.89	32.66 ± 2.25	33 ± 2.36	32.36 ± 1.81	30.33 ± 1.86	40.33 ± 2.25	39.96 ± 1.66
LDL	57.13 ± 0.67	51.79 ± 2.22	53.57 ± 1.57	48.17 ± 2.37	47.18 ± 1.84	42.59 ± 1.578	53.69 ± 1.54	56.55 ± 0.68
VLDL	25.99 ± 0.5	24.04 ± 0.58	28.8±6.14	33 ± 2.36	31.03 ± 2.35	27.74 ± 0.281	26.99 ± 0.86	23.8 ± 2.28

Data represented as mean ± SD (n = 6). Statistical significance was tested by one-way ANOVA ($P < 0.05$) followed by Bonferroni multiple comparison tests. ALP = alkaline phosphatase, AST = aspartate transaminase, ALT = alanine transaminase, HDL = high density lipoprotein, LDL = low density lipoprotein, VLDL = very low-density lipoprotein.

monitored and documented (Table 5, Fig. 1 and 2). All groups exhibited weight gain compared to their initial weights. Additionally, the percentage of body weight gain decreased with increasing dosage in both the treated male and female groups compared to the control group (Table 6, Fig. 3).

3.7 Hematological parameters

Hematological parameters like WBC, RBC and the differential cell counts were almost similar and no considerable difference was identified in LSLE-treated groups (Table 7) and other parameters were also in the permissible physiological range.

3.8 Biochemical analysis

Biochemical parameters for kidney and liver function, including ALP AST, ALT, albumin, bilirubin, globulin, protein, triglycerides, creatinine, HDL, LDL and VLDL, showed no significant changes with an incremental increase in the doses of LSLE administration in comparison with the male and female control groups. However, the level of glucose and pancreatic amylase recorded specific changes in both the treated male and female groups (Table 8).

3.9 Relative organ weight and histopathology

The important internal organs (kidney, liver, heart, lungs and spleen) isolated from the different groups showed no abnormalities upon gross examination, nor were there any significant variations in mean weight between the treated and control groups (Table 9). Histological analysis of the kidney, liver, heart, lungs and spleen revealed no pathological changes following treatment, even at the highest dosage of 400 mg of LSLE administered over 90 days (Fig. 4).

3.10 Phytochemical analysis

LCMS analysis of LSLE showed molecular ion peak $(M + H)^+$ at (1) M/Z 473.2 (Retention time-4.270 min, (2) M/Z 172 (Retention time - 4.270 min and (3) M/Z 455.1(Retention time- 13.341 min. (Fig. 5 A, B). Subsequently, the advanced chromatography analysis (HPLC) revealed the constituents as corosolic acid, gallic acid and oleanolic acid at 0.17, 0.082 and 0.85 %, respectively (Fig. 6 A - F).

Discussion

The growing interest in natural plant products as alternatives to synthetic pharmaceuticals has led to a significant rise in their demand (34). The assertion that these products are risk-free must be validated only through modern scientific screening methods, including comprehensive short-term and long-term toxicity studies of the plant products (35). Toxicological assessments are carried out on different experimental animals to evaluate the safety of natural products for human use. These tests help determine the product's efficacy and establish criteria for determining safe dosages for humans. Moreover, it is challenging to identify specific adverse effects in animals, such as. headaches, visual disturbances, dizziness and abdominal pain. Additionally, interspecies differences in pharmacokinetic parameters present another obstacle for accurately extrapolating the effects to human. In our acute toxicity studies, doses up to 3000 mg/kg did not lead to any adverse effects or abnormal habits within the first 6 h. Also, no mortality was detected over the 14-day experimental period. Primarily, studies across a wide range of doses must be performed to optimize an adequate dosage for sub-chronic and chronic toxicity studies, which should be equal to or exceed the recommended human dose. Therefore, short-term and long-term toxicity studies are performed to assess the

Table 5. Impact of chronic oral administration of the LSLE (mg/kg/day) on body weight, food intake and water intake of Wistar rats

Week (w)	Normal (Male)	Normal (Female)	100 (Male)	100 (Female)	200 (Male)	200 (Female)	400 (male)	400 (Female)
Body weight (g)								
M - 0	150 ± 1.78	145 ± 0.89	156.66 ± 1.36	160.66 ± 2.06	157 ± 2.68	148 ± 2.36	162 ± 1.78	155 ± 0.89
M1 - 30 days	171.66 ± 1.03	164 ± 0.89	179.33 ± 1.36	180.33 ± 0.51	175 ± 0.89	163.33 ± 1.36	180.33 ± 1.36	173.66 ± 1.36
M2 - 60 days	192.66 ± 1.36	184.33 ± 1.86	195 ± 1.78	199.33 ± 2.25	191 ± 0.89	179.66 ± 1.36	194 ± 2.68	186.33 ± 1.36
M3 - 90 days	225.66 ± 1.36	216.66 ± 1.36	220.66 ± 1.36	216.66 ± 1.36	209.66 ± 1.86	207 ± 0.89	208.66 ± 2.58	205.66 ± 1.03
Food intake (gm/day/rat)								
W1	21.66 ± 0.51	21.53 ± 0.45	22.7 ± 0.88	22.52 ± 0.48	22.9 ± 0.4	21.81 ± 0.29	22.32 ± 0.81	23.52 ± 0.22
W2	21.46 ± 0.45	21.43 ± 0.45	21.47 ± 0.43	21.77 ± 0.18	22.11 ± 0.74	21.06 ± 0.44	21.51 ± 0.43	21.33 ± 0.83
W3	20.52 ± 0.45	19.96 ± 0.34	20.85 ± 0.22	20.98 ± 0.78	21.43 ± 0.45	19.78 ± 0.34	21.11 ± 0.54	20.41 ± 0.64
W4	19.57 ± 0.26	19.8 ± 0.39	19.9 ± 0.49	20.15 ± 0.13	20.73 ± 0.67	19.2 ± 0.79	20.74 ± 0.16	20.46 ± 0.39
W5	19.16 ± 0.2	19.38 ± 0.17	20.18 ± 0.2	19.68 ± 0.1	19.97 ± 0.21	19.02 ± 0.1	20.4 ± 0.26	20.28 ± 0.12
W6	19.18 ± 0.27	19.193 ± 0.14	19.73 ± 0.16	19.38 ± 0.1	19.55 ± 0.11	18.83 ± 0.21	19.55 ± 0.17	19.89 ± 0.25
W7	19.44 ± 0.33	19.16 ± 0.13	19.27 ± 0.1	19.05 ± 0.04	19.22 ± 0.08	18.71 ± 0.39	19.09 ± 0.08	19.34 ± 0.27
W8	19.3 ± 0.24	19.24 ± 0.25	18.98 ± 0.08	18.67 ± 0.25	19.08 ± 0.09	18.19 ± 0.26	18.98 ± 0.15	19.04 ± 0.26
W9	18.9 ± 0.18	19.36 ± 0.31	18.81 ± 0.22	18.72 ± 0.15	18.5 ± 0.18	17.71 ± 0.18	18.49 ± 0.18	18.93 ± 0.13
W10	18.59 ± 0.33	19.01 ± 0.19	18.58 ± 0.24	18.56 ± 0.31	18.49 ± 0.41	17.8 ± 0.21	18.3 ± 0.45	18.49 ± 0.27
W11	18.5 ± 0.22	18.82 ± 0.21	18.25 ± 0.19	18.55 ± 0.75	18.16 ± 0.23	17.74 ± 0.24	17.84 ± 0.38	18.31 ± 0.18
W12	18.46 ± 0.31	18.89 ± 0.46	17.89 ± 0.35	19 ± 0.27	17.54 ± 0.51	17.32 ± 0.1	17.68 ± 0.31	17.82 ± 0.31
Water intake (mL/day/rat)								
W1	22.43 ± 1.11	21.59 ± 1.26	22.66 ± 0.59	22.94 ± 0.61	22.6 ± 0.44	22.39 ± 0.58	23.13 ± 0.85	22.05 ± 0.86
W2	22.62 ± 0.88	22.46 ± 0.42	22.13 ± 0.8	22.16 ± 1.03	21.83 ± 0.66	22.41 ± 0.46	22.85 ± 0.63	22.91 ± 0.74
W3	21.31 ± 0.17	21.7 ± 0.34	21.54 ± 0.6	21.74 ± 1.27	21.27 ± 0.74	21.61 ± 0.43	21.88 ± 0.97	21.41 ± 0.58
W4	20.93 ± 0.53	20.84 ± 0.59	21.01 ± 0.83	21.96 ± 0.71	21.51 ± 0.38	21.11 ± 0.51	21.45 ± 1.08	20.79 ± 0.43
W5	21.01 ± 0.29	20.92 ± 0.4	20.74 ± 0.5	21.29 ± 0.75	20.81 ± 0.84	20.63 ± 0.35	21.05 ± 1.07	20.96 ± 0.11
W6	20.84 ± 0.43	21.1 ± 1.14	21.25 ± 0.42	20.8 ± 0.75	21.12 ± 0.33	21.12 ± 0.45	21.34 ± 0.6	20.35 ± 0.2
W7	20.27 ± 0.36	20.5 ± 0.87	20.11 ± 0.45	20.26 ± 0.74	20 ± 0.74	20.03 ± 0.3	20.68 ± 0.48	19.86 ± 0.39
W8	19.63 ± 0.38	20.41 ± 0.89	20.34 ± 0.75	19.92 ± 0.57	19.83 ± 0.62	19.49 ± 0.43	19.62 ± 0.63	19.54 ± 0.29
W9	20.51 ± 0.54	20.25 ± 0.83	20.29 ± 0.85	20.05 ± 0.42	19.96 ± 0.51	19.62 ± 0.54	19.65 ± 0.38	19.7 ± 0.3
W10	19.4 ± 0.54	19.69 ± 0.14	19.88 ± 0.09	19.55 ± 0.4	19.36 ± 0.46	19.55 ± 0.42	19.43 ± 0.33	19.46 ± 0.36
W11	19.86 ± 0.5	19.69 ± 0.58	19.26 ± 0.11	19.48 ± 0.51	19.47 ± 0.34	19.56 ± 0.19	19.15 ± 0.15	18.8 ± 0.34
W12	19.74 ± 0.59	19.72 ± 0.52	19.48 ± 0.25	18.93 ± 0.54	18.8 ± 0.62	19.3 ± 0.05	18.79 ± 0.19	18.7 ± 0.3

Data represented as mean ± SD (n = 6). Statistical significance was tested by two-way ANOVA ($P < 0.05$) followed by Bonferroni multiple comparison tests (M = month, W = week).

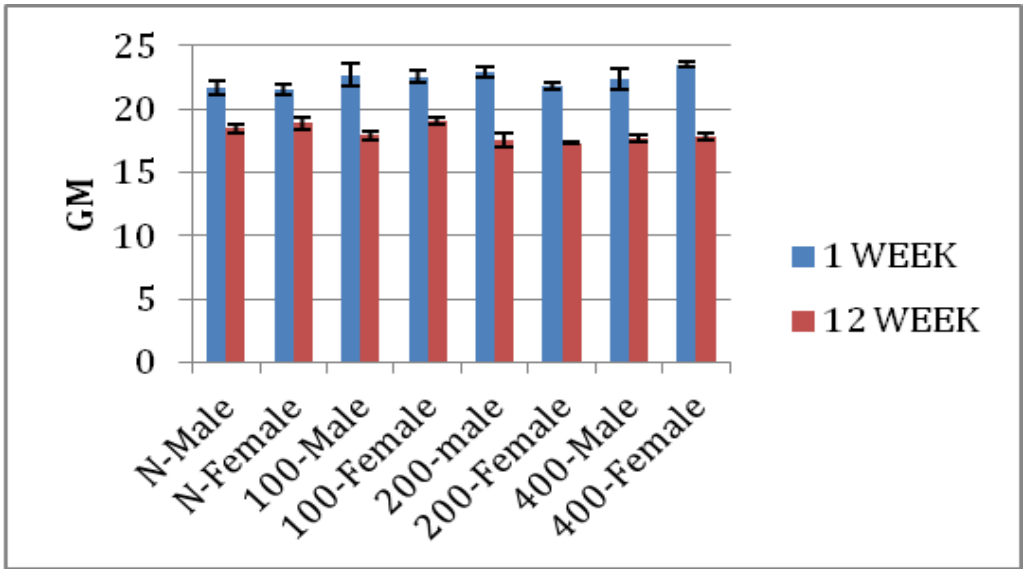

Fig. 1. Percentage reduction in food intake in the Wister rats after 90 days of treatment with LSLE.

Values are expressed as mean ± SD of 6 values, statistical significance was tested using two-way ANOVA (P < 0.05) followed by Bonferroni multiple comparition tests.

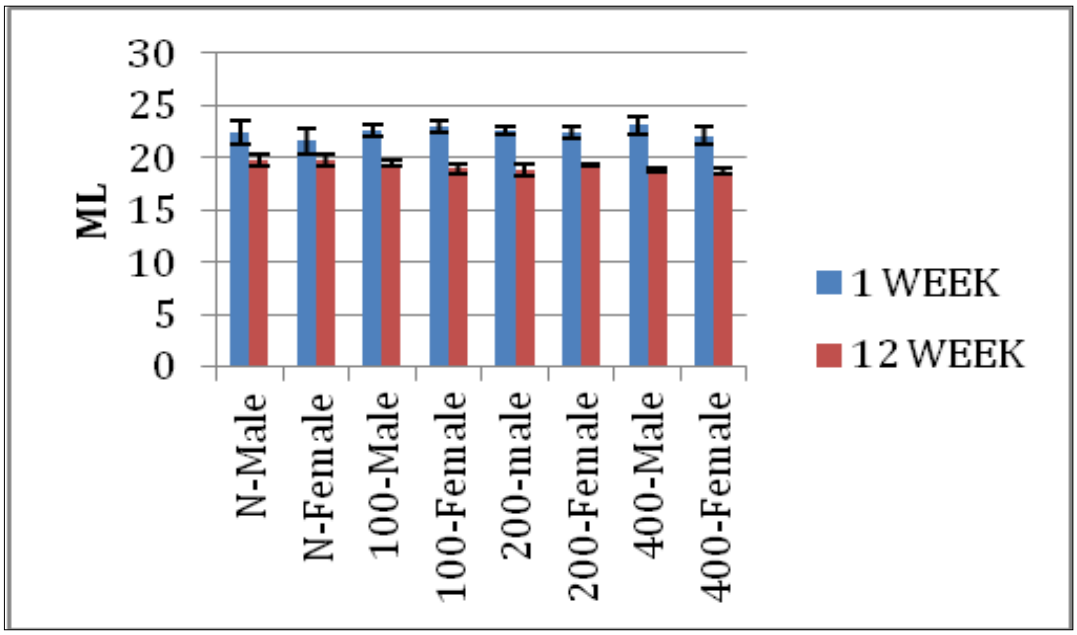

Fig. 2. Percentage reduction in water intake in the Wister rats after 90 days of treatment with LSLE. Values are expressed as mean ± SD of 6 values, statistical significance was tested using a Two-way ANOVA ($P < 0.05$) followed by Bonferroni multiple comparition tests.

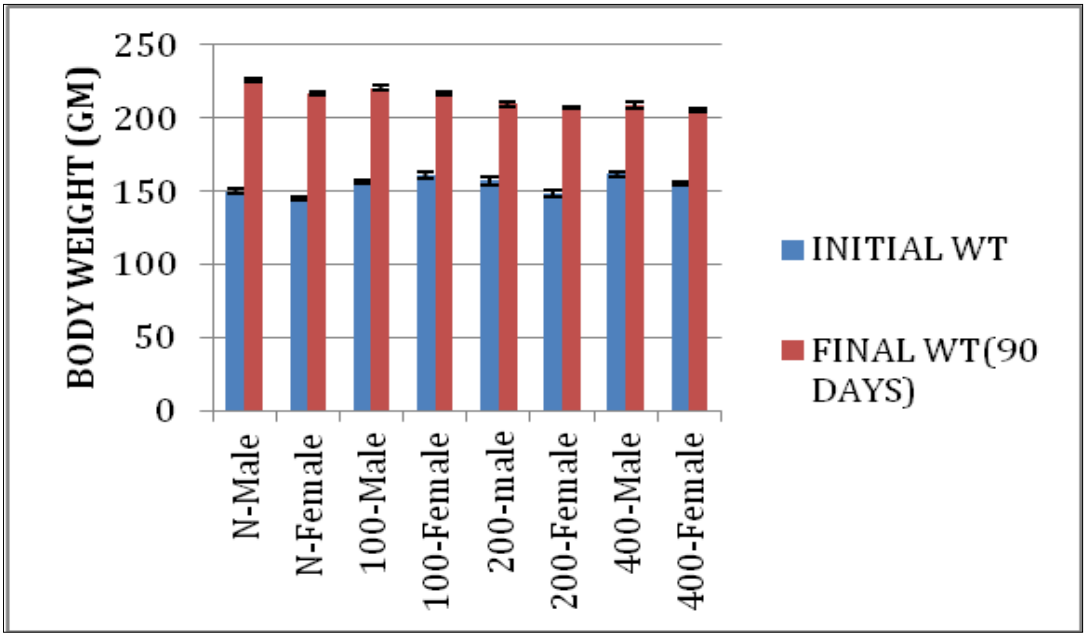

Fig. 3. Percentage reduction of body weight in Wistar rats after 90 days of treatment with LSLE.

Values are expressed as mean ± SD of 6 values, statistical significance was tested using a two-way ANOVA $p \leq 0.05$ followed by Bonferroni multiple comparison tests.

Table. 6. Percentage increase in body weight of Wistar rats following 90 days of treatment with LSLE

Groups (90 Days)	Weight gain (%)
N - Male	50.44 ± 1.36
N - Female	49.42 ± 1.37
100 - Male	40.85 ± 1.35
100 - Female	34.85 ± 1.36
200 - Male	33.54 ± 1.86
200 - Female	39.89 ± 0.89
400 - Male	28.81 ± 2.58
400 - Female	32.68 ± 1.03

Data represented as mean ± SD (n = 6). Statistical significance was tested using two-way ANOVA ($P < 0.05$).

Table 7. Impact of chronic oral dosing of LSLE (mg/kg/day) on the hematological parameters in male and female Wistar rats

Parameter	Normal (Male)	Normal (Female)	100 (Male)	100 (Female)	200 (Male)	200 (Female)	400 (male)	400 (Female)
RBC ($X10^6$/µL)	7.51 ± 0.14	7.09 ± 0.09	7.13 ± 0.26	7.01 ± 0.25	7.25 ± 0.17	6.97 ± 0.194	7.05 ± 0.28	6.84 ± 0.2
HG (g/dL)	13.88 ± 0.2	13.8 ± 0.29	14.09 ± 0.37	13.51 ± 0.1	14.09 ± 0.19	13.93 ± 0.18	14.1 ± 0.27	13.73 ± 0.34
MCV (fL)	50 ± 0.002	62 ± 0.001	60 ± 0.002	60 ± 0.003	60 ± 0.002	60 ± 0.001	61 ± 0.001	63 ± 0.002
MCH (pg)	18.4 ± 0.026	19.3 ± 0.05	19.6 ± 0.04	19.2 ± 0.06	19.4 ± 0.07	20 ± 0.032	19.9 ± 0.09	20 ± 0.032
MCHC (g/dL)	31.56 ± 0.77	31.01 ± 0.25	31.46 ± 0.95	31.03 ± 0.72	32.03 ± 0.22	30.79 ± 0.22	31.76 ± 1	31.46 ± 0.64
WBC ($X10^3$/µL)	3.04 ± 0.29	2.89 ± 0.12	3.16 ± 0.16	2.9 ± 0.25	3.3 ± 0.16	2.93 ± 0.27	3.12 ± 0.09	2.88 ± 0.096
Neautrophyl (%)	16.35 ± 1.48	14.65 ± 0.55	16.35 ± 0.83	14.74 ± 0.67	16.28 ± 0.65	15.24 ± 0.47	16.43 ± 0.68	15.54 ± 0.49
Lymphocytes (%)	74.66 ± 2.25	73.76 ± 0.83	75.83 ± 1.96	73.97 ± 1.53	75.11 ± 2.09	73.43 ± 2.39	75.3 ± 1.18	73.9 ± 1.93
Monocytes (%)	5.25 ± 0.51	5.04 ± 0.3	4.91 ± 0.64	4.71 ± 0.55	5.53 ± 0.44	4.64 ± 0.72	5.49 ± 0.48	4.99 ± 0.38
Eosinophyl (%)	1.96 ± 0.29	1.62 ± 0.29	1.9 ± 0.2	1.45 ± 0.22	1.86 ± 0.16	1.51 ± 0.33	1.78 ± 0.34	1.26 ± 0.22

Data are represented as the mean ± SD (n = 6). Statistical significance was tested using one-way ANOVA ($P < 0.05$) followed by Bonferroni multiple comparison tests. RBC = red blood cells, HG = hemoglobin, MCV = mean corpuscular volume, MCH = mean cell hemoglobin, MCHC = mean corpuscular hemoglobin concentration, WBC = White blood cells.

Table 8. Effect of long-term oral dosing of the LSLE (mg/kg/day) on biochemical parameters of Wistar rats

Parameter	Normal (Male)	Normal (Female)	100 (Male)	100 (Female)	200 (Male)	200 (Female)	400 (male)	400 (Female)
Albumin	4.74 ± 0.41	4.4 ± 0.18	4.93 ± 0.48	4.6 ± 0.4	4.97 ± 0.27	4.59 ± 0.09	5.32 ± 0.47	4.9 ± 0.12
ALP	2.94 ± 0.13	2.73 ± 0.06	2.81 ± 0.27	2.54 ± 0.2	3.04 ± 0.37	2.61 ± 0.23	2.93 ± 0.3	2.67 ± 0.14
Bilirubin	1.77 ± 0.05	1.7 ± 0.04	1.79 ± 0.049	1.7 ± 0.06	1.74 ± 0.07	1.67 ± 0.04	1.82 ± 0.06	1.73 ± 0.06
Creatinine	0.42 ± 0.04	0.4 ± 0.07	0.46 ± 0.05	0.4 ± 0.05	0.48 ± 0.04	0.33 ± 0.05	0.42 ± 0.05	0.38 ± 0.03
Globulin								
Glucose	116 ± 3.22	112.33 ± 5.08	112.66 ± 6.28	108 ± 3.22	114.5 ± 3.44	107.66 ± 5.08*	112.33 ± 3.14*	106.16 ± 6.52*
Pancreatic amylase	2.25 ± 0.16	2.08 ± 0.03	2.42 ± 0.19	2.35 ± 0.08	2.4 ± 0.12	2.13 ± 0.14	2.41 ± 0.23**	2.36 ± 0.18**
Protein	7.33 ± 0.13	6.71 ± 0.57	7.43 ± 0.37	6.81 ± 0.23	7.16 ± 0.14	6.72 ± 0.17	6.72 ± 0.17	6.79 ± 0.34
AST	93 ± 2.36	87.33 ± 3.38	91 ± 7.79	92 ± 3.89	97.66 ± 2.73	92 ± 2.68	93.33 ± 4.03	89.66 ± 6.94
ALT	33.66 ± 1.36	29.33 ± 1.86	31 ± 3.57	30.66 ± 2.58	34.33 ± 2.73	28.66 ± 1.86	32.33 ± 1.86	30.33 ± 1.86
Urea	36.8 ± 1.43	33.33 ± 1.36	40 ± 1.78	37 ± 1.78	38 ± 2.68	35.1 ± 1.65	40.66 ± 2.73	37 ± 3.22
Triglycerides	136.66 ± 3.38	138 ± 4.73	146 ± 10.88	147 ± 3.09	146.33 ± 9	145.66 ± 5.46	147.66 ± 15.75	150 ± 11.83
HDL-Cholesterol	32.66 ± 1.86	32.66 ± 1.86	37.33 ± 0.51	33.66 ± 1.36	36.33 ± 1.36	33 ± 1.54	38 ± 5.86	35.33 ± 3.72
LDL-Cholesterol	57.66 ± 2.58	47.4 ± 2.57	49.13 ± 6.39	41.6 ± 4.97	56.66 ± 7.72	51.86 ± 3.35	46.46 ± 13.22	44.33 ± 10.67
VLDL-Cholesterol	27.33 ± 0.67	27.6 ± 0.94	29.2 ± 2.17	29.4 ± 0.61	29 ± 1.89	29.13 ± 1.093	29.53 ± 3.15	30 ± 2.36

Data are represented as the mean ± SD (n = 6). Statistical significance was assessed using one-way ANOVA ($P < 0.05$), followed by the Bonferroni multiple comparison test. ALP = alkaline phosphatase, AST = aspartate transaminase, ALT = alanine transaminase, HDL = high-density lipoprotein, LDL = low-density lipoprotein, VLDL = very low-density lipoprotein.

Table 9. Impact of chronic oral administration of the LSLE (mg/kg/day) on organ weight of Wistar rats

Organs	Normal (Male)	Normal (Female)	100 (Male)	100(Female)	200 (Male)	200 (Female)	400 (male)	400 (Female)
Heart	0.85 ± 0.02	0.8 ± 0.01	0.91 ± 0.05	0.89 ± 0.02	0.85 ± 0.05	0.85 ± 0.04	0.9 ± 0.03	0.86 ± 0.02
Kidney	1.65 ± 0.02	1.64 ± 0.01	1.72 ± 0.01	1.74 ± 0.03	1.71 ± 0.02	1.67 ± 0.03	1.75 ± 0.01	1.68 ± 0.05
Liver	5.2 ± 0.08	5.09 ± 0.08	5.35 ± 0.05	5.19 ± 0.09	5.31 ± 0.25	5.17 ± 0.25	5.33 ± 0.09	5.23 ± 0.12
Lungs	1.76 ± 0.02	1.73 ± 0.02	1.87 ± 0.01	1.81 ± 0.01	1.79 ± 0.08	1.76 ± 0.01	1.82 ± 0.02	1.78 ± 0.05
Spleen	0.82 ± 0.01	0.81 ± 0.02	0.87 ± 0.01	0.85 ± 0.01	0.85 ± 0.08	0.81 ± 0.02	0.85 ± 0.02	0.84 ± 0.01

Data are represented as mean ± SD (n = 6). Statistical significance was tested using one-way ANOVA ($P < 0.05$).

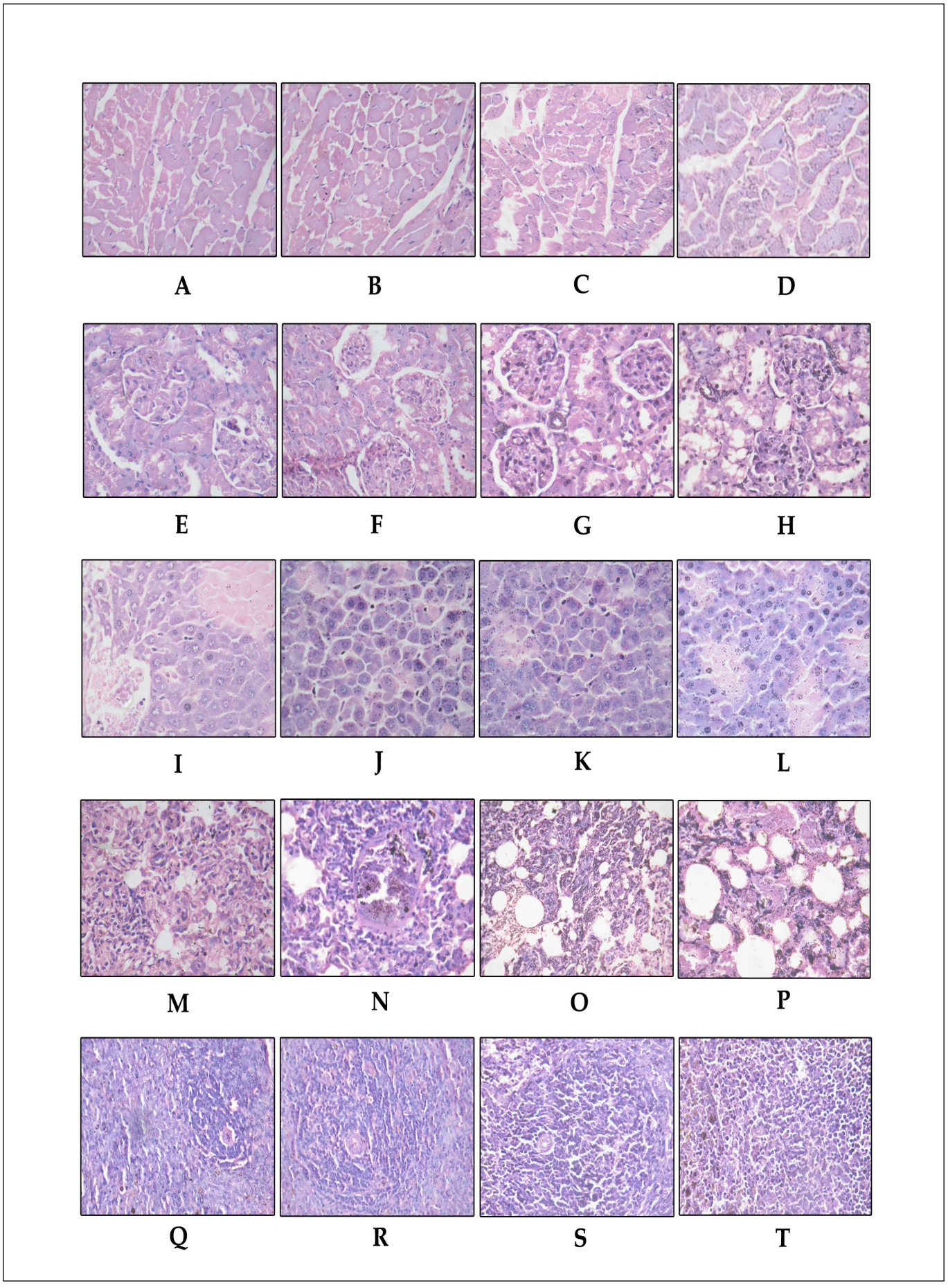

Fig. 4. Photomicrographs of rat tissues stained with hematoxyline and eosin (100X).

Heart (A) control, (B) 100, (C) 200 and (D) 400 mg/Kg LSLE-treated rat, **Kidney** (E) control, (F) 100, (G) 200 and (H) 400mg/Kg LSLE-treated rat, **Live**r (I) control, (J) 100, (K) 200 and (L) 400 mg/Kg LSLE -treated rat, **Lungs** (M) control, (N) 100, (O) 200 and (P) 400mg/Kg LSLE-treated rat, **Spleen** (Q) control, (R) 100, (S) 200 and (T) 400 mg/Kg LSLE-treated rat.

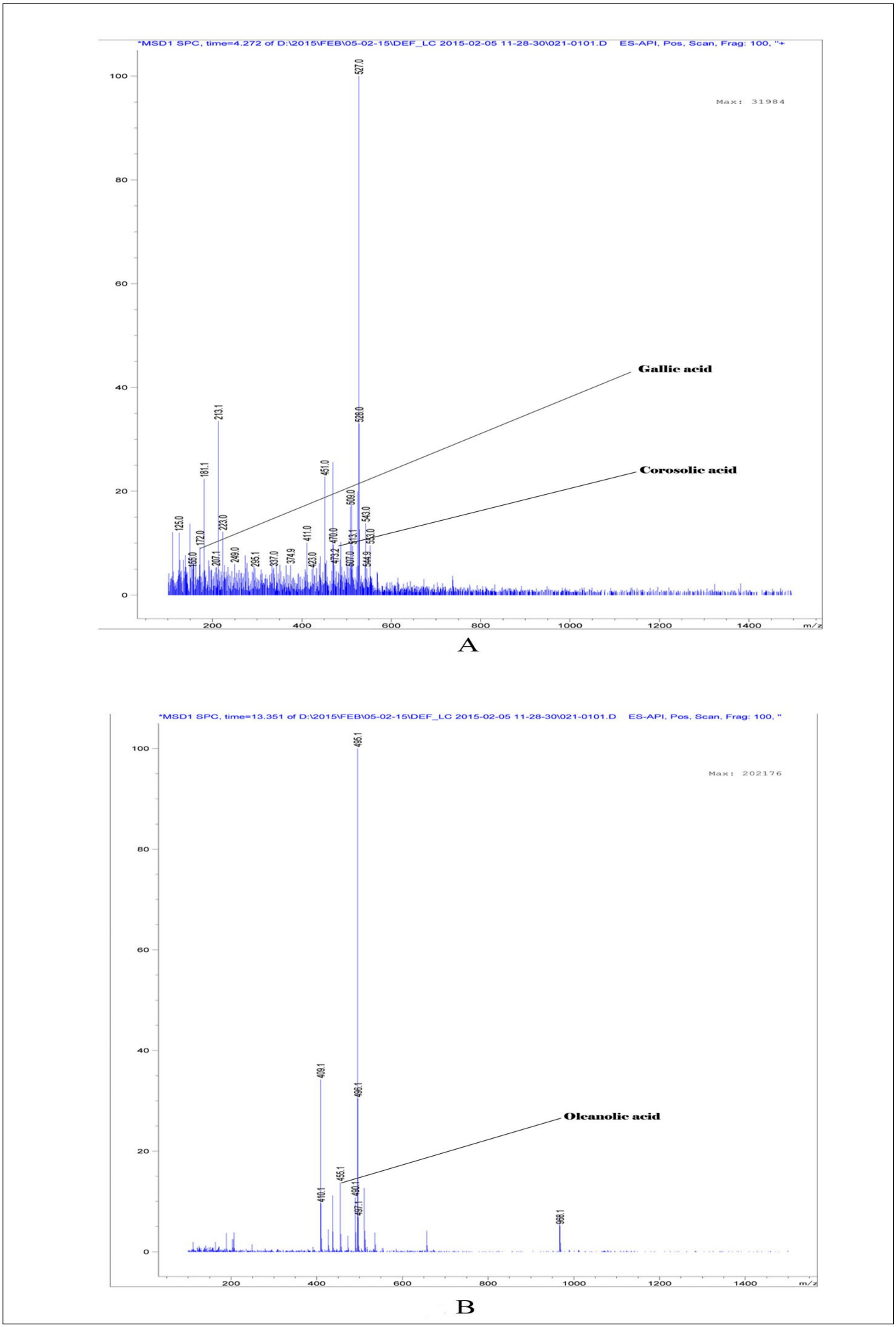

Fig. 5. LCMS chromatograms of LSLE. Identification of peaks. A-Gallic acid, Corosolic acid, B -Oleanolic acid.

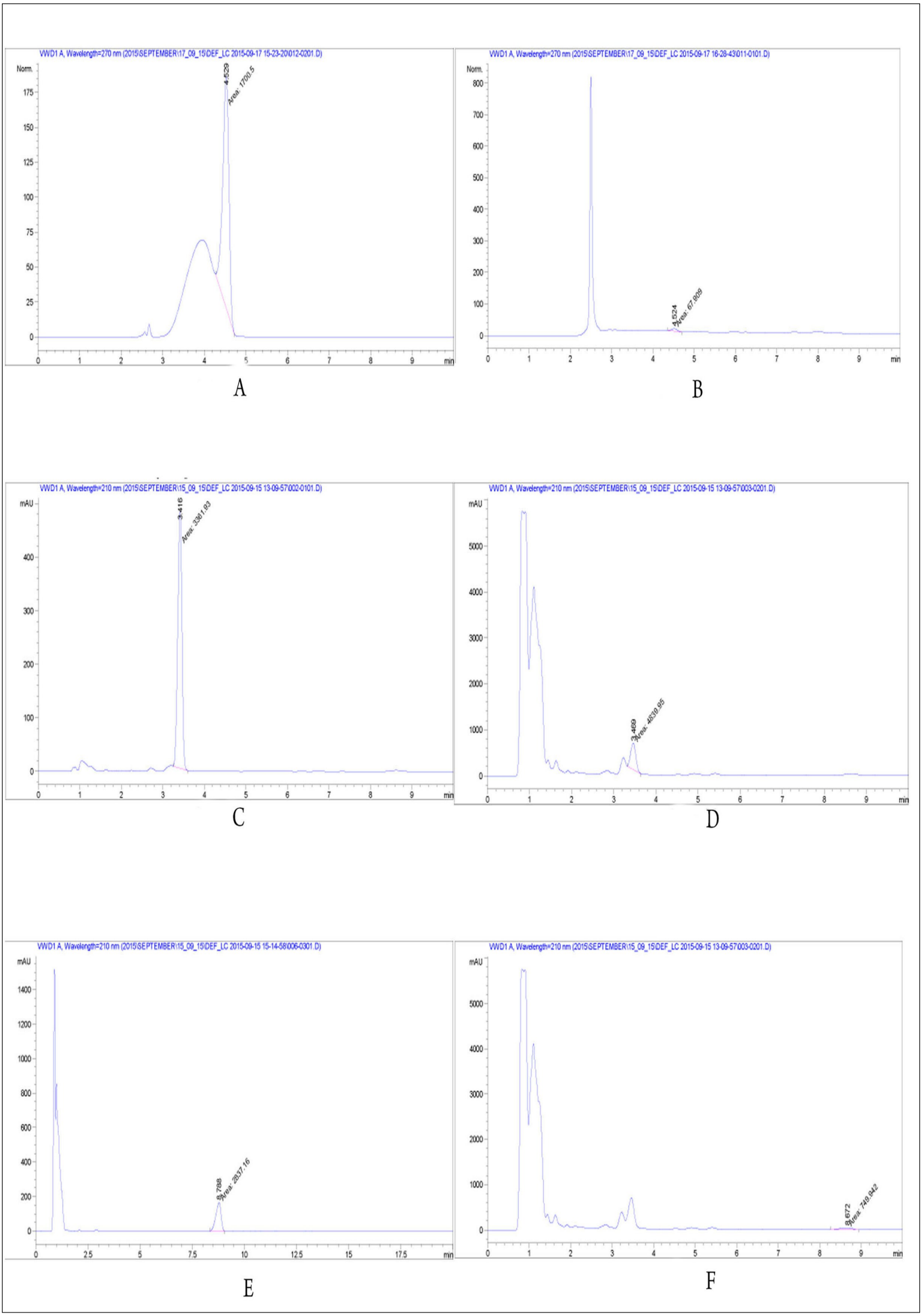

Fig. 6. HPLC chromatograms of LSLE. A- std Gallic acid, B-sample, C-std Corosolic acid, D-sample, E-std Oleanolic acid, F -sample.

drawbacks of a drug or natural product after extended use. These studies offer insights into potential health risks associated with continuous exposure over a brief period, including information about the target organ affected. The 28-day short-term and 90-day long-term toxicity studies revealed that LSLE administered at doses of 100, 200 and 400 mg/kg administered daily, did not cause any fatalities or exhibit clinical symptoms of toxicity. Body weight and relative organ weights in both control and treated animal groups are commonly used to assess the toxic effects or potential adverse outcomes of natural products or drugs. The present studies indicate no significant alteration in organ weight between 2 sets of animals.

Loss of body weight less than 10% of initial weight indicates adverse effects of the study material (36). In our sub-chronic and chronic studies, however, neither group lost weight during the treatment. The findings indicate a significant difference in the percentage of weight gain after both experiments. A notable decrease in food consumption, commonly believed to contribute to the observed reduction in body weight and appetite loss, is often linked to weight loss resulting from disturbances in the metabolism of carbohydrates, proteins, or fats. This could potentially explain the weight loss observed in the current study (37). The notable phenomenon of both the short and long-term toxicity studies was the intake of food and water. The reduction in food intake is consistent with previous findings that demonstrate the blood sugar lowering effect of *L. speciosa* on alloxan-induced diabetic mice (38). Previous reports have shown that biologically active substances with therapeutic potential obtained from *L. speciosa*, including the pentacyclic triterpene acids, may also play a prominent role in reducing the percentage of body weight gain (39, 40). The long-term toxicity study also shows a significant reduction in the percentage of body weight gain evaluated against the control groups of both sex and hence agreeable with the earlier observation of (41). It is presumed that LSLE may ameliorate visceral adiposity and, therefore, possess anti-obesity potential by influencing carbohydrate and fat metabolism. Furthermore, corosolic acid suppresses adipogenesis and stimulates lipolysis, thereby helping to reduce body weight gain and the accumulation of adipose tissue. It also appears to regulate lipid metabolism by decreasing triglyceride levels while increasing HDL cholesterol, highlighting its potential in addressing lipid imbalances associated with obesity (42).

Furthermore, blood profile assessment is also crucial, as it serves as a sensitive indicator of noxious substances and provides a vital pointer to pathological and physiological states in both humans and animals (43). The stability of hematological and biochemical parameters observed in the LSLE-treated groups can be attributed to the administration of LSLE. The kidney, one of the most essential organs, is susceptible to various factors that can impair its function and is often a target for the hidden toxic effects of drugs, which can ultimately lead to renal failure (44). Since it is widely recognized that most drugs, chemicals and xenobiotics are cleared through renal discharge, it became essential to assess the impact of the extracts on kidney function (45). The stable levels of renal biochemical markers, such as urea and creatinine, indicate that LSLE does not cause any imbalance in kidney function.

The levels of protein, bilirubin, albumin, triglycerides, HDL, LDL and VLDL were measured to evaluate the overall biochemical profile of the experimental animals and identify any metabolic changes. The results from both short-term and long-term experiments indicated no significant differences between the treated and control groups of either sex.

To examine the liver function AST, ALT and ALP were assessed and the values of these markers were found to be near the values of the control groups. These markers are good indicators of liver functions (46). Any damage to the liver's parenchymal cells would have resulted in an increase in the levels of both transaminases in the bloodstream (47). AST in the serum originates from both mitochondrial and cytoplasmic sources and any rise in its levels can be considered an early indicator of cell damage, resulting in the release of enzymes into the serum (48). The glucose and pancreatic amylase levels were significantly altered in the treated groups of both short-term and long-term experiments compared to the control groups of both sexes. Previous studies, animal models reported the effect of corosolic acid in lowering the blood glucose level and the pancreatic amylase inhibitory effect may be due to the synergic effect of the triterpene acids, especially oleanolic acid and corosolic acid (49, 50).

Histopathological examinations of the internal organs such as the kidney, liver, spleen, heart and lungs did not indicate any morphological variations post administration of the LSLE at various doses in both experiments.

From the preliminary screening (LCMS), it is observed that the molecular mass corresponds respectively to the active constituents such as corosolic acid, gallic acid and oleanolic acid. The present results coincide with the previous report on L.S (51). The significant products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxylnonenal (4 HNE) (51). MDA induces oxidative stress and lead to the formation of advanced lipoxidation end products (ALE). MDA serves as a biomarker to measure the oxidative stress level. From our previous investigation, we found that LSLE may prevent these deleterious effects by inhibiting lipid peroxidation, thus reducing the formation of MDA. As LCMS (scan mode) is a more sophisticated method for the evaluation of compounds, the molecular weight of the known compounds in LSLE was identified from the previously reported studies and the peaks corresponding to these molecules in LCMS were compared with the identified molecules (52). Among these compounds, corosolic acid, gallic acid and oleanolic acid showed similar molecular weight. The presence of these identified compounds was subsequently validated by HPLC using authentic standards. The present study identified a few active compounds in LSLE, which includes mainly the pentacyclic triterpenoids and antioxidant compounds. The efficacy of LSLE may, therefore, be attributed to the complex mixture of the triterpenoids as mentioned above and antioxidant compounds.

The safety of oral LSLE administration aligns with previous studies, where acute and sub-acute toxicity were evaluated (53). In the acute study, a single oral dose of 2000

mg/kg of LS was administered, whereas, in the sub-acute study, a daily dosage of 200 mg/kg was given for 28 days. Furthermore, the prospective study of LSLE extract in lowering blood glucose levels and treating Type 2 diabetes was demonstrated in (54). Interestingly, the oral administration of an even higher dosage of LSLE (400 mg/kg/day) for short and long-term use in experimental rats of both sexes did not initiate any other biochemical, hematological and histopathological evidence of toxicity. The current investigation, therefore, indicates that LSLE is relatively safe for consumption as a dietary supplement or herbal drug.

Conclusion

In light of these findings, no notable adverse effects were observed in the groups treated with LSLE at various doses for 28 days and 90 days of duration. This study validates the non-toxicity of LSLE over both short- and long-term periods, revealing no significant changes in hematological, histopathological, or other biochemical parameters. Nevertheless, additional studies are necessary to assess different parameters, such as neurotoxicity, carcinogenicity, teratogenicity, mutagenicity and genetic parameters to complement the safety usage of this plant. Further, clinical evaluation is necessary to accurately determine the safe dosage for humans because human toxicity may not always be accurately forecasted from animal studies.

Data availability

The data underlying this article are provided as supplemental figures and tables in the paper and are also available on request.

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Authors' contributions

SJS did the experimental work under the research supervision of SSR and PPP. The statistical analysis of the raw data was checked and analysed by KS. The draft manuscript prepared by SJS was refined and finalized by KS and PPP.

Compliance with ethical standards

Conflict of interest: The Authors’ do not have any conflict of interests to declare.

Ethical issues: None

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