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Research Article
Safety Assessment of *Schleichera oleosa* Lour. Leaves Extract: Acute and Subchronic Studies

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Abstract

Background and Objective: *Schleichera oleosa* (Sapindaceae) has been reported to be useful in traditional medicine and it has some potential pharmacological activities, such as anticancer, antioxidant and antimicrobial activities. This study aimed to assess its safety to provide complete data required for the development of *S. oleosa* as herbal medicine. **Materials and Methods:** The safety assessment of the extract was carried out by testing acute and subchronic toxicity in mice (male and female) and rats (male and female), respectively. The doses used in the acute toxicity test were 1000, 2000, 3000, 4000 and 5000 mg kg^{-1} of body weight and those in the subchronic treatment were 100, 200 and 400 mg kg^{-1} of body weight. **Results:** In the acute toxicity test, the *S. oleosa* leaf extract at all doses indicated that the LD_{50} value of the extract was higher than 5000 mg kg^{-1} b.wt., which suggested that this extract is practically non-toxic according to the toxicity criteria. Furthermore, the subchronic toxicity test found that the administration of the extract to male and female rats at a daily dose of 100 and 200 mg kg^{-1} b.wt., for 90 days did not cause any significant change in blood haematology, blood biochemistry and histopathological picture of liver, kidney, heart, lymph and lung. Despite there being a significant increase in white blood counts, long-term use of the *S. oleosa* leaf extract is relatively safe. **Conclusion:** The results provided evidence regarding the potential of *S. oleosa* leaves to be used as herbal medicine. However, further research needs to be done to verify that activity and its safety in long-term use.

Key words: *Schleichera oleosa* L., extract, herbal medicine, toxicity, 50% lethal dose, acute, subchronic

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Schleichera oleosa L. is a member of the Sapindaceae family and is often used by the community as a medicinal plant. In Indonesia, *S. oleosa* can grow well on Java, Bali, East Nusa Tenggara, Sulawesi and the Maluku Islands. In South Sulawesi, the smoke from the burned, dry leaves of the *S. oleosa* tree is used as a treatment (fumigation) for skin diseases. The plant's seed parts are typically processed to produce kusum oil, also known as Makassar oil, which is traditionally used to treat itching, acne, burns and other skin problems and rheumatism and as a hair growth agent[1].

Schleichera oleosa leaves contain several phytochemical components in the form of alkaloids, phenolics, tannins and flavonoids, with the highest levels being phenolics and flavonoids[1]. Due to their phenolic and flavonoid content, *S. oleosa* leaves have a strong antioxidant capacity. One type of antioxidant, based on its mechanisms, is phenolic compounds and their derivatives including flavonoids which cover a large phytochemical group in plants[2]. Flavonoids have been shown to have antioxidant activity, free radical scavenging capacity and they also help prevent oxidative stress[3]. The mechanism of flavonoids as antioxidants involves directly scavenging reactive oxygen species (ROS), preventing ROS regeneration and indirectly increasing the antioxidant activity of cellular antioxidant enzymes. Flavonoids prevent the formation of ROS in several ways, including inhibiting the activity of xanthine oxidase and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase enzymes, as well as chelating metals (Fe^{2+} and Cu^{2+}) to prevent redox reactions that can produce free radicals[4,5].

Some research results show the pharmacological effects of *S. oleosa*, including antioxidant, antibacterial and anticancer activities[6]. Phenolic acid functions as a medicinal antioxidant that lowers levels of free radicals, lower exposure to toxic metals and modifies gene expression and cell signaling pathways. The high content of polyphenols can ward off free radicals and prevent cell damage. *Schleichera oleosa* has strong antioxidant activity due to its ability to scavenge free radicals, which is related to its cytotoxic effect, indicating its toxicity to various cell lines. It was found to be an effective anticancer agent. In addition, the research results also show its potential as an effective antimicrobial agent because it shows good activity against various types of microbes[7].

In the development of medicinal preparations or traditional medicines, apart from having evidence of efficacy, it is also required to test for toxicity in experimental animals to ensure safety when used in humans, both acute and long-term (subchronic) testing. Toxicity assessment of herbal medicines aims to identify side effects and determine the exposure level at which these effects occur[8]. Additionally, animal toxicity studies help investigate any biochemical, physiological, or pathological reactions that may occur before human use[9]. Acute and subchronic toxicity data are necessary to ensure human safety[10,11] and risks of toxicity and side effects severely limit the use of herbs in therapeutic settings[12]. Toxicity data are therefore expected to increase consumer confidence in the safety of drugs under development[13].

This research was conducted to prove the long-term safety (subchronic) of ethanol extract of *S. oleosa* leaves in experimental animals *in vivo*. It is hoped that the research results can reveal the level of safety of ethanol extract of *S. oleosa* leaves and form the basis of subsequent security testing.

MATERIALS AND METHODS

Study area: This research was conducted at the Pharmacology and Clinical Pharmacy Laboratory, Faculty of Pharmacy, Padjadjaran University, Indonesia, from October, 2020 to June, 2021.

Materials: The materials used in this study was the *S. oleosa* leaves, ethanol 70% (Merck®), Rotary Evaporator (IKA®-Malaysia), Pulvis Gummi Arabici (Food Grade®), EDTA Vacutainer tube (BD Vacutainer®), Vortex (Thermo Scientific™ Type: 88880018), centrifuge (Thermo Fisher SKU: 75002421), Microtome (Leica RM 2235), Microscope (Olympus CX33).

Extract preparation: *Schleichera oleosa* L. was obtained from South Sulawesi. *Schleichera oleosa* leaves were determined at the Indonesian Institute of Sciences, Biology Research Center, Botany-LIPI Bogor (No: 726/IPH.1.01/If.07/VII/2020). This determination was made to ensure the precise identity of the plants used in the study.

The leave powder was extracted using 70% ethanol as a solvent using the maceration method[14]. The simplicia was placed in the maceration container, followed by the addition of a sufficient solvent for the wetting process. The remaining solvent was added until all simplicia was completely submerged and then allowed to stand in a place protected from sunlight for three days with occasional stirring. Following the filtration, the residue was macerated with the same solvent. The extract was concentrated in a rotary evaporator (IKA®-Malaysia) at 60°C, 75 rpm to obtain a thick extract. The extract was stored at 4°C until used.

Animals: For toxicity acute testing, male and female mice strains of Swiss Webster (n = 60) weighed 25-30 g (male) and 25-30 g (female) had been used. For subchronic toxicity assessment, the healthy 2-3 months old male and female rats strain of Wistar (n = 30), weighing 200-250 g were used. The animals were obtained from the School of Life Science and Technology, Bandung Institute of Technology, Indonesia. Before treatment, the animals were adapted for one week and provided with adequate food and drink under a 12 hrs light/dark cycle at room temperature. All experimental procedures were approved with the Padjadjaran University Research Ethics Committee (498/UN6.KEP/EC/2020).

Research procedure

Phytochemical screening: The phytochemical screening was carried out based on the standard method as described in the former study[15] to identify secondary metabolites in the extracts including alkaloids, flavonoids, terpenoids, steroids, saponins, phenolic compounds and tannins.

Acute toxicity study: Acute toxicity study of ethanol extract of *Schleichera oleosa* L.(EESL) has been subjected to male and female white mice (*Mus musculus*) strains Swiss Webster. Male and female mice were divided into six groups, each group consisted of five mice. The group was divided into a controlled solvent (only given 1% Pulvis Gummi Arabici (PGA) suspension) and five treatment groups, which were given various doses of the extract (1000, 2000, 3000, 4000 and 5000 mg kg^{-1} b.wt.). Daily observation of rat mortality was conducted for 14 days. In addition, behavioral patterns including changes in physical appearance, body weight and other physiological activities and signs of toxicity were observed daily during extract administration for 14 days.

Subchronic toxicity study: Rats were divided into six groups, where each group consisted of five male or five female rats. The group consisted of a control group (CG: Given 2% PGA suspension), a test group of dose I (DG 100: Given 100 mg kg^{-1} b.wt., EESL), a test group of dose II (DG 200: Given 200 mg kg^{-1} b.wt., EESL) and a test group of dose III (DG 400: Given 400 mg kg^{-1} b.wt., EESL). The lowest level was 100 mg kg^{-1} b.wt., which is the effective dose showing hepatoprotective activity. Control satellite group (CSG: Given only 2% PGA suspension) and satellite group (CS400: Given 400 mg kg^{-1} b.wt., EESL). The EESL were given orally for 90 consecutive days, according to the group. For the satellite group, after 90 days of EESL administration, the rats were kept alive and were maintained without administration of EESL until 120 days. Evaluation of subchronic toxicity assessments included observation of body weight, animal organ indexes, determination of hematological and biochemical parameters of the animals' blood and examination of histopathology of animals' organs.

Histopathology: Histopathological analysis was carried out at the Laboratory of Animal Biosystems, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University using the HE (Hematoxylin Eosin) staining technique. Organs were washed with 0.9% NaCl, fixed in a 10% formalin solution, then dehydrated in alcohol, embedded in paraffin and sliced using a microtome (Leica RM 2235) with a thickness of 6-8 µm. The sections were stained with hematoxylin and eosin and then observed under the microscope (Olympus CX33). Quantitative observations of each organ include the number of normal cells, necrosis, hydropic degeneration and fatty degeneration. Qualitative observations were made by observing the state of the central vein, sinusoids, bleeding and inflammatory cell infiltration.

Statistical analysis: Statistical analysis using SPSS 24. Statistical significance was determined by One-way Analysis of Variance (ANOVA) and the *post hoc* least significant difference (LSD) test. The p-values of less than 0.05 were considered significant.

RESULTS

Phytochemical screening: *Schleichera oleosa* L. leaves extracted by the maceration method. Phytochemical screening aims to determine the presence of a class of secondary metabolite compounds present in the extract and can be a qualitative description of the content of the extract. The results of the phytochemical screening showed that regardless of its origin, the ethanolic extract of *S. oleosa* leaves contain alkaloids, flavonoid and steroid phenol.

Acute toxicity study: The number of dead mice after administration of leaf extract *S. oleosa* had been observed after the administration of 1000, 2000, 3000, 4000 and 5000 mg kg^{-1} bwt., doses (Fig. 1a-b). Another parameter that had been observed were body weight and signs of toxicity after daily administration for 14 days.

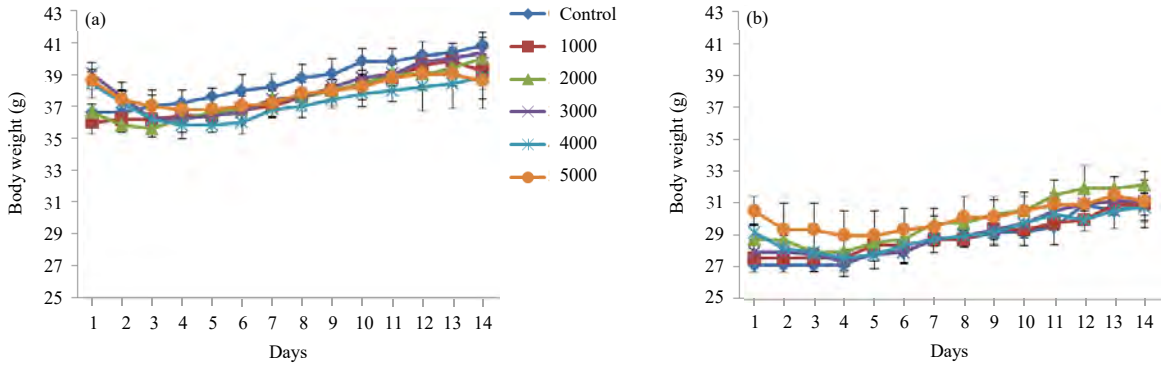


Fig. 1(a-b): Average body weight of (a) Male and (b) Female mice during acute toxicity study (n = 5)

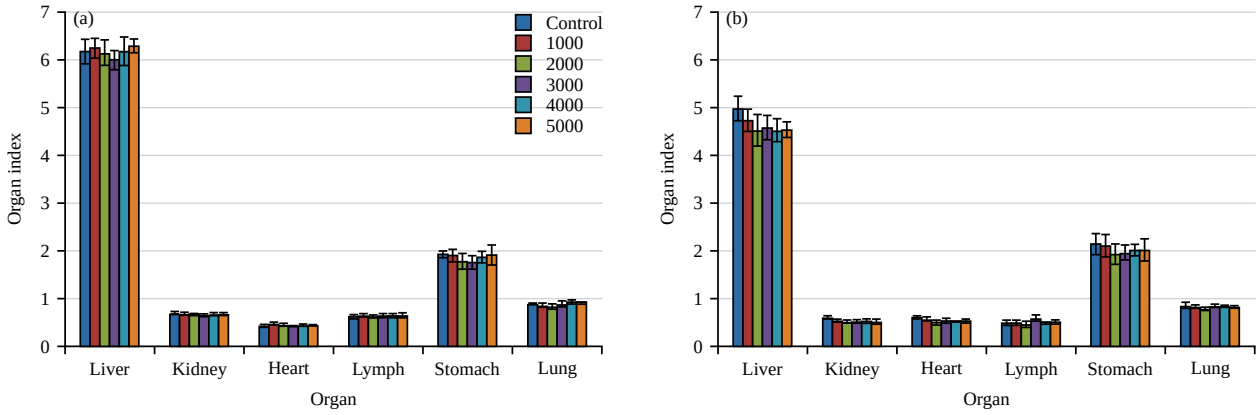


Fig. 2(a-b): Organ index of (a) Male and (b) Female mice after acute toxicity test of *S. oleosa* leaf ethanolic extract

At the initial time after extract administration, symptoms appeared in several mice at doses of 4000 and 5000 mg kg^{-1} bwt., as decreased movement activity and frequent sleep compared to those from controls. Starting day 3rd, the sleep symptom had disappeared and the activity has come back to normal. Signs of toxicity (skin, hair and eyes) from all the groups showed normal responses. Mortality was not found in either male or female group, until 14 days of observation.

Observations on the changes in several animals' organs after treatment had been assessed as a sensitive indicator of the toxic effects caused by new substances or compounds in experimental animals. Based on the observations, after EESL administration, the weight of several organs of the mice, including the liver, kidney, heart, lymph, stomach and lungs was observed and the index of these organs was calculated (Fig. 2a-b). Furthermore, statistical analysis was carried out to see the differences between groups.

Subchronic toxicity study: A subchronic toxicity study of EESL was conducted to determine the level of safety of EESL for long-term use, referring to the provisions of non-clinical tests. The test aims to obtain information on the presence of toxic effects of EESL that were not detected in the acute toxicity test, information on the possibility of toxic effects after repeated exposure to the EESL within a certain period and information on doses that do not cause effects. Observation of body weight of male and female rats during a subchronic toxicity test (Fig. 3a-b).

To find out more about the effect of giving EESL, observations were also made on several male and female rat organs, consisting of the liver, kidney, heart, lymph and lungs. The determination of organ index after treatment is used as an indicator to determine the effect of the induced compound on experimental animals and their effects on organs (Fig. 4a-b).

The hematological examination is needed to establish the diagnosis and to monitor the toxicity in animals during subchronic toxicity assessment. The results were compared with those from the control group (Table 1). Blood biochemical parameters of the animals after subchronic toxicity assessment were presented in Table 2.

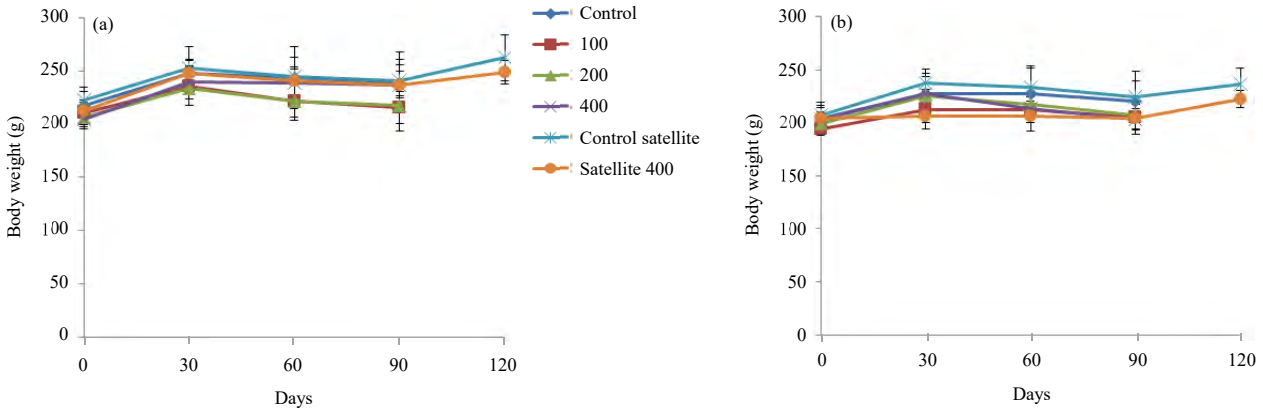


Fig. 3(a-b): Average body weight of (a) Male and (b) Female rats during 120 days of observation (n = 5)

Table 1: Hematological parameters in male and female rats

		Dose group (mg kg^{-1} b.wt.)			Satellite (mg kg^{-1} b.wt.)	
Parameter test	Control (CG)	DG 100	DG 200	DG 400	Control (CSG)	400 (CS400)
Male						
WBC (×10^3/µL)	6.85±1.18	7.58±1.10	9.52±1.01	10.42±0.58	7.37±0.85	10.02±2.37
LYM (×10^3/µL)	3.13±0.32	3.33±0.21	3.5±0.24	3.54±0.28	3.56±0.28	3.6±0.17
RBC (×10^6/µL)	5.38±0.04	5.71±0.26	5.85±0.69	6.19±0.24	5.7±0.09	5.53±0.25
Hb (g dL^{-1})	16.25±0.68	16.32±0.32	16.92±0.27	17.06±0.47	16.53±0.38	16.57±0.32
HCT (%)	43.17±2.51	47.73±4.51	49.03±2.86	51±3.11	47.63±1.49	47.65±1.18
PLT (×10^3/µL)	500±45.92	500±29.82	515±24.56	519.3±67.50	460.3±53.31	488.7±40.82
Female						
WBC (×10^3/µL)	6.65±0.58	7.67±1.00	8.65±1.12	8.48±1.10	6.05±1.24	7.09±0.91
LYM (×10^3/µL)	3.09±0.29	3.3±0.20	3.68±0.56	3.92±0.71	3±0.54	3.43±0.20
RBC (×10^6/µL)	4.31±0.19	5.11±0.45	5.36±0.55	6.85±0.61*	5.33±0.14	4.84±0.90
Hb (g dL^{-1})	14.77±0.61	15.31±0.44	15.56±1.10	15.84±1.42	15.65±0.46	15.61±0.71
HCT (%)	47.03±4.77	48.14±1.21	49.77±1.27	50.13±2.21	46.73±0.21	48.01±1.54
PLT (×10^3/µL)	445.7±25.72	469.3±7.37	503±23.43	521.3±34.2*	418.3±36.47	488.3±17.62

WBC: White blood cells, LYM: Lymphocytes, RBC: Red blood cells, Hb: Hemoglobin, HCT: Hematocrit and PLT: Platelet, *p<0.05 significant when compared to the control group and ±: Standard deviation

Table 2: Blood biochemistry parameters in male and female rats

		Dose group (mg kg^{-1} b.wt.)			Satellite (mg kg^{-1} b.wt.)	
Parameter test	Control (CG)	DG 100	DG 200	DG 400	Control (CSG)	400 (CS400)
Male						
SGOT/AST (U L^{-1})	165.7±21.08	186±16.09	203.3±8.02	217.7±13.87	174.7±18.58	193.7±12.50
SGPT/ALT (U L^{-1})	61.3±4.04	73.3±4.73	71.7±6.51	70.0±2.65	57±2.65	72±7.81
Urea (mg dL^{-1})	39.2±3.50	45.6±7.91	44.4±4.23	51.8±6.67	41.8±2.21	47.6±2.02
Creatinine (mg dL^{-1})	0.44±0.08	0.43±0.05	0.45±0.04	0.48±0.04	0.49±0.04	0.42±0.04
Female						
SGOT/AST (U L^{-1})	167±5.00	186.7±19.09	197.7±17.16	192.7±10.97	162±9.17	202.3±14.01
SGPT/ALT (U L^{-1})	52±3.00	59.7±4.51	63.7±2.52	62.3±3.06	55.3±3.51	58.0±5.29
Urea (mg dL^{-1})	37.5±5.17	41.9±2.90	37.6±9.75	48.1±8.52	39.5±4.80	44.9±2.56
Creatinine (mg dL^{-1})	0.51±0.11	0.53±0.09	0.51±0.06	0.51±0.06	0.47±0.08	0.51±0.06

SGOT: Serum glutamate oxaloacetate transaminase, AST: Aspartate aminotransferase, SGPT: Serum glutamate pyruvic transaminase, ALT: Alanine aminotransferase, and ±: Standard deviation

Measurement results from male and female rats (Table 2) showed the liver health parameters, namely SGOT and SGPT. There was no significant difference in SGOT and SGPT levels between the do groups either in male or female rats compared with those from the control (p>0.05). Observation of kidneys' health data, namely urea and creatinine levels, showed that the values of these two parameters either in male or female rats were not significantly different between the

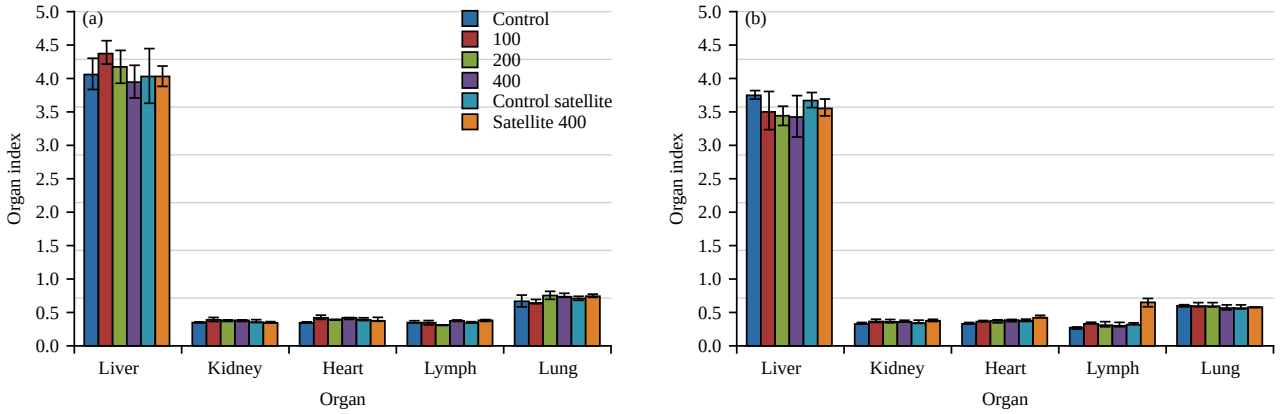


Fig. 4: Organ index of (a) Male and (b) Female rats after subchronic toxicity test of *S. oleosa* leaf ethanolic extract

EESL dose groups compared with the control group ($p>0.05$). This finding was supported by histopathological features which revealed that no changes in liver or kidney tissue had been founded.

The results of histopathological observations showed no changes in the liver, kidneys, heart and lungs for all dose groups, except inflammation which was only found in the lymph in the highest dose group (400 mg kg^{-1} bwt.) in both males and females (Fig. 5), which then recovered to normal after 30 days of recovery (satellite group). This condition indicated that increasing the quantity of EESL can cause inflammation in specific organs during administration and the effect disappeared after it had been cut off.

DISCUSSION

In this study, acute and subchronic toxicity of the *S. oleosa* leaf extract was tested. The acute toxicity test was conducted to determine the short-term adverse effects of the extract when administered in a single dose for 24 hrs in animals. The LD_{50} value, defined as the dose of a chemical that kills 50% of test animals, was used as an index of a drug's acute toxicity[9,16]. The subchronic study was carried out to assess the adverse effects of the extract being given repeatedly over a period to test animals. This study was used to provide information on target organ toxicity and identify possible side effects of the extract[17].

In the acute toxicity test, the *S. oleosa* leaf extract in the acute toxicity test, the *S. oleosa* leaf extract did not result in the death of any experimental animals over the 14 days observation period at any of the doses utilized (1000, 2000, 3000, 4000 and 5000 mg kg^{-1}) in 14 days observation. This indicated that the LD_{50} value of the extract was higher than 5000 mg kg^{-1}, which suggested that this extract is practically non-toxic. This assumption was based on the results of Kennedy's study, which defines toxicity categories for chemicals from highly toxic to virtually non-toxic, counting LD_{50} values above 5000 mg kg^{-1} as virtually non-toxic[18]. Furthermore, a non-toxic effect of the extract during the acute toxicity test was also observed, indicated by a non-significant difference observed in the change of body weight and organ weight compared to the control and no unwanted effect in animal behaviours.

In the subchronic toxicity test, there was no significant disturbance in the growth of the body weight of animals, although, after 30 days of exposure to the extract, there was a slight decrease in body weight, but, it was also shown in the control animals. After 120 days of extract exposure (the satellite group), the body weight increased normally like those in the satellite control animals. This indicated that the *S. oleosa* leaf indicated that the *S. oleosa* leaf extract didn't affect animal metabolism.

As shown in Fig. 4a-b there was no discernible difference in the internal organ weight of the animals after repeated administration of the *S. oleosa* extract at the three doses for 90 days to the male and female mice. Additionally, there was no discernible difference in the weight of the internal organs (liver, heart, kidney, lymph and lung) between the sexes of the animals. Throughout the extract treatment like that observed in the control group, suggesting that the *S. oleosa* leaf extract was not toxic to the mentioned organs. These results were supported by the histopathological data that showed no changes in the liver, kidneys, heart and lung features in all doses of the *S. oleosa* extract, although inflammation of the lymph was found in the highest dose (400 mg kg^{-1}), but, it recovered to normal after 30 days of recovery (Fig. 5).

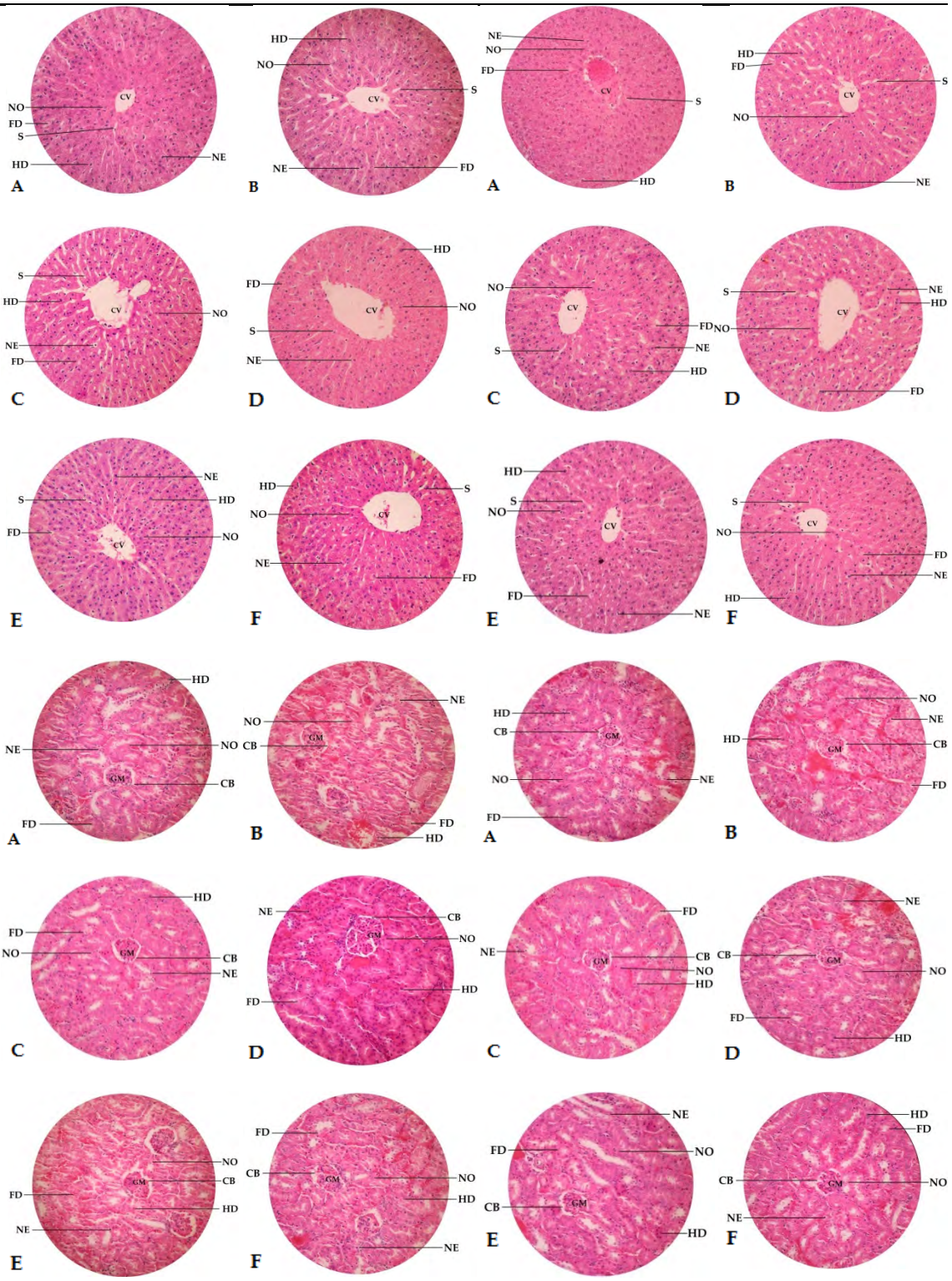
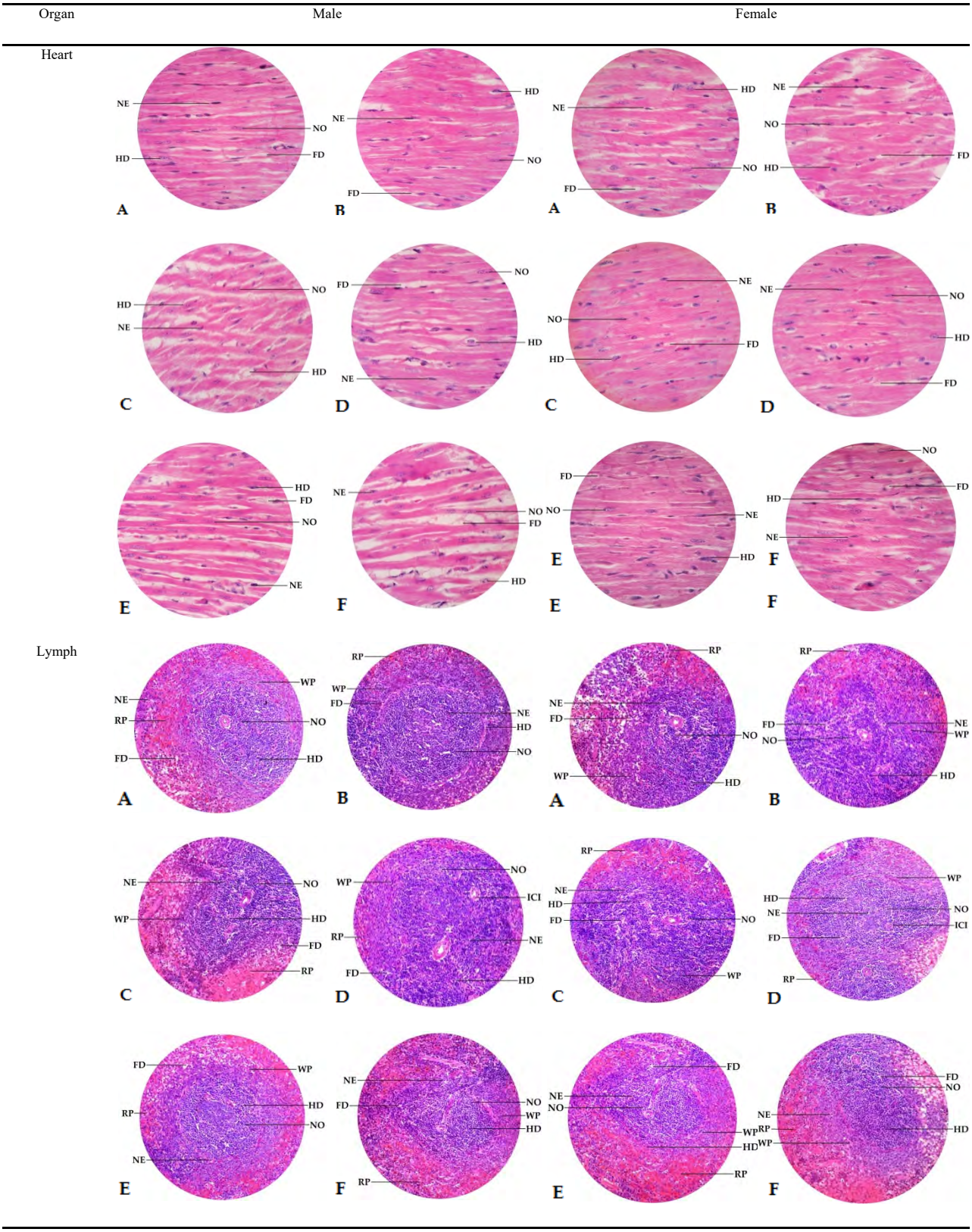
Organ	Male	Female
Liver		
Kidney		

Fig. 5(a-f): Continue



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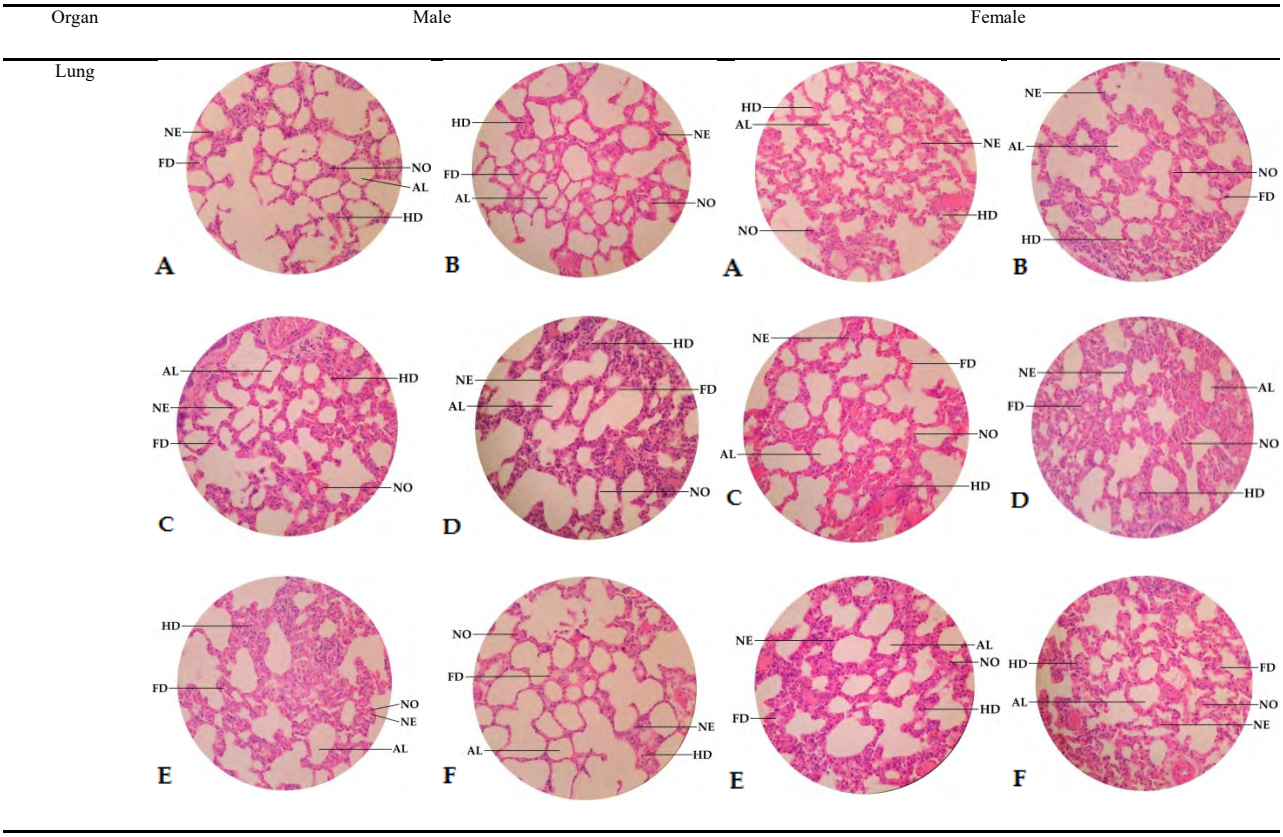

Fig. 5(a-f): Histopathological features of organs of Control group (CG), Dose group of EESL, DG 100, 200, 400 mg kg^{-1} b.wt., Satellite control (CSG) and satellite 400 mg kg^{-1} b.wt. (CS400) with HE staining, A: Control group, B: Dose of 100 mg kg^{-1} b.wt., C: Dose of 200 mg kg^{-1} b.wt. D: Dose of 400 mg kg^{-1} b.wt., E: Satellite control and F: Satellite dose of 400 mg kg^{-1} b.wt.)

AL: Alveolus, CB: Capsule Bowman's, CV: Central vein, FD: Fatty degeneration, GM: Glomerulus, HD: Hydropic Degeneration, ICI: Inflammatory cell infiltration, NE: Necrosis, NO: Normal, RP: Red pulp, S: Sinusoid and WP: White pulp

In the observation on hematological parameters, after a repeated administration of the three doses of the extract, no significant difference was found in the counts of haematological parameters as compared to the control, except for the white blood counts, which were increased significantly ($p<0.05$) in the male animals. A significant increase ($p<0.05$) was also observed in the satellite groups at a dose of 400 mg kg^{-1} compared to the satellite control, but it happened only in the male animals. This might be evidence of the undesirable effects of the *S. oleosa* leaf extract on haematological parameters in the subchronic doses.

Evaluation of liver and kidney function after daily administration of EESL for 90 days which had been carried out to assess the toxicity of the extract was based on their role as vital organs in living organisms[19]. The parameters measured were SGOT, SGPT, urea and creatinine. Changes in SGOT and SGPT values are sensitive indices to indicate the degree of liver cell damage[19]. The SGOT and SGPT values will be released from liver cells into the bloodstream when liver cells are damaged or chronic liver injury, increasing serum content. In the citric acid cycle, SGOT and SGPT enzymes catalyse the transfer of amine groups from glutamate to produce the amino acids alanine and aspartate[20,21]. The SGOT and SGPT levels that exceed the control indicate a disturbance to the integrity of hepatocyte cells. If there is damage to hepatocytes, SGOT and SGPT will be released into the bloodstream. Upon examination of liver and kidney function, statistical analysis showed that no significant changes had been found in all groups after 90 days of administration of EESL compared with those in the controls group.

Examining biochemical blood parameters, SGOT, SGPT, urea and creatinine levels were measured as parameters for liver and kidney disease. An increase in SGOT and SGPT values are sensitive indicators of liver cell damage, whereas a high level in creatinine and urea is a sensitive index to indicate the presence of impaired kidney function. The impaired kidney

function causes a decrease in the rate of renal filtration, accompanied by the accumulation of metabolic wastes (urea and creatinine) in the blood so that the levels of these two substances will increase[22,23]. Urea is a product of nitrogen metabolism or protein catabolism. Creatinine is a type of amino acid that is a waste product in the blood and is excreted through the kidneys into the urine. High creatinine levels are thought to be due to strenuous muscle activity or an impaired renal excretory system[24,25]. In this study, SGPT, urea and creatinine levels were at normal levels, which were not significantly different from those in the control group. However, the SGOT level in both sexes of animals increased significantly from the control at the three doses used and the increase also occurred in the satellite group (Table 2). The high level of SGOT indicated that toxicity may occur in the liver after extract exposure for 90 days, but this raises a question because the SGPT value remains normal.

According to the literature, SGPT is a cytosolic enzyme found in high concentrations in the liver, which is a specific parameter of liver injury. Unlike SGOT, it is neither sensitive nor liver-specific and increased SGOT can also be considered as a secondary non-hepatic cause. The SGOT is found not only in the liver but also in cardiac muscle, skeletal muscle, kidney, brain, pancreas, lung, white blood cells and red blood cells[26]. So, high SGOT levels obtained in this study may be due to the effect of the extract on other organs after long-term administration of the extracts. However, this issue certainly needs further research.

According to the acute toxicity test, the extract was categorized as practically non-toxic and the long-term use of the extract at effective doses was relatively safe. The *S. oleosa* plant offers potency to be used as a medicinal. However, it still needs further studies to verify the activity and to obtain more complete evidence concerning this activity.

In future research, it is recommended to carry out special toxicity tests, such as toxicity tests for teratogens, mutagens and carcinogens, to obtain more complete data regarding the toxicity profile of *S. oleosa* extract for further development in the form of medicinal preparations.

CONCLUSION

According to the acute toxicity test, the extract was categorized as practically non-toxic and the long-term use of the extract at effective doses was relatively safe. The *S. oleosa* plant offers potency to be used as a medicinal. However, it still needs further studies to verify the activity and to obtain more complete evidence concerning this activity.

SIGNIFICANCE STATEMENT

In the development of medicinal preparations or traditional medicines, apart from having evidence of efficacy, it is also required to test for toxicity in experimental animals to ensure safety when used in humans, both acute and long-term (subchronic) testing. Toxicity testing in animals is helpful to see whether there are biochemical, physiological and pathological reactions that may arise before use in humans. This research was conducted to prove the long-term safety (subchronic) of ethanol extract of *S. oleosa* leaves in experimental animals *in vivo*. It is hoped that the research results can reveal the level of safety of ethanol extract of *S. oleosa* leaves and form the basis of subsequent security testing.

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