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Original Article

Acute Oral Toxicity Assessment of Freeze-Dried Lipote Fruit Extract (*Syzygium polycephaloides* (C. B. Rob.) Merr.) in ICR Mice

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ABSTRACT

Introduction: Lipote (*Syzygium polycephaloides* (C. B. Rob.) Merr.) has been traditionally used in Ayurvedic medicine due to its nutritional and bioactive contents. **Methods:** An acute oral toxicity test was conducted following the OECD 425 guidelines to investigate the toxic effects of freeze-dried lipote fruit extract (LFE) in male and female ICR mice at doses of 55, 175, 550, 2000, and 5000 mg/kg BW. **Results:** At the end of the 14-day experimentation period, no physical, behavioral, neurologic, or cardiorespiratory signs of toxicity nor mortalities were recorded in LFE-treated mice. Also, physiologic parameters such as body weight, and feed and water intake registered normal throughout the study. Hematologic values such as total RBC, total WBC, and differential WBC for both sexes remained normal, apart from the male mouse administered with 2000 mg/kg LFE dose which presented erythrocytopenia, leukocytopenia, and lymphocytopenia after the end of the experimentation period, most likely due to extraneous factors unrelated to treatment. Meanwhile, the blood creatinine and blood urea nitrogen values remained within their respective normal reference ranges. **Conclusion:** It can be inferred from results of this acute oral toxicity study that LFE is relatively non-toxic, has an LD_{50} above 5000 mg/kg, and like other closely related *Syzygium* berries, does not elicit any adverse effects on the physiologic, hematologic, and blood chemical levels of kidney-filtered substances in mice. Sub-chronic and chronic toxicity studies must be conducted to determine the safety of continuous oral ingestion of lipote fruit.

Key words: Acute toxicity, Lipote, Mice, Philippine berry, Safety.

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INTRODUCTION

Lipote (*Syzygium polycephaloides* (C. B. Rob.) Merr.) is an indigenous berry under the Myrtaceae family that is widely cultivated in parts of Northern and Southern Luzon in the Philippines.[1,2] It is a small-to-medium tree bearing a compact cluster of small, round, red to purple fruits, which are commonly processed into jams, juices, and wines.[3] Lipote is traditionally used in Ayurvedic medicine for the treatment of diabetes mellitus, high blood cholesterol, hypertension, and cough and is found to be enriched in proteins, fats, carbohydrates, crude fiber, minerals such as calcium, phosphorus, iron, and β-carotene, and vitamins A, B_1, B_2, B_6 and C.[4] Moreover, it contains biologically active phenolic compounds such as flavonoids, phenolic acids, delphinidin-3-glucoside, ellagic acid, quercetin, and rutin, as well as, non-phenolic components including oleanolic acid, ursolic acid, β-sitosterol, and squalene.[5,6] A gram of fresh lipote fruit has a total phenolic content of 2,780 μg gallic acid equivalent (GAE) and a total flavonoid content of 5,141 μg quercetin equivalent (QE).[5]

Previous studies have reported the remarkable biological properties of lipote including antimicrobial,[7] antifungal,[8] blood cholesterol-lowering,[6,9] blood pressure-lowering,[6] and blood glucose-lowering activities.[10,11] It also exhibits tumor suppressing activity by inhibiting malignant transformation, inducing cancer cell apoptosis[12,13] and impeding tumor cell growth.[14] Additionally, it displays significant antioxidant activity that is equipotent to those natural and synthetic antioxidants such as vitamin E, butylhydroxyanisole (BHA), and butylhyroxytoluene (BHT), by retarding membrane lipid peroxidation and by enhancing site- and non-site-specific scavenging activities.[5]

However, despite these accumulating health-enhancing benefits and anecdotal evidence of long-term consumption in the countryside, there has been no toxicity studies documented to date concerning this indigenous lipote berry. Therefore, an acute oral toxicity test of freeze-dried lipote fruit extract (LFE) was conducted using sexually mature ICR mice of both sexes, following the OECD 425 up and down method (OECD, 2008).[15] Specifically, this study aimed to determine the clinical signs of toxicity, morbidity and mortality, as well as, physiologic, behavioral, hematological, and blood chemical changes as a prelude to the development of this berry as a high-value supplement or as a functional food ingredient.

MATERIALS AND METHODS

Plant collection and preparation of LFE

Fully-riped lipote fruits were harvested from selected areas in Laguna, Philippines. Prior to processing, samples were submitted to the Botanical Herbarium, Museum of Natural History, University of the Philippines - Los Baños (UPLB) for authentication. The fruits were then deseeded at room temperature using a pulper (Kiya Keisakusho, Japan) and underwent freeze-drying at 20 °C and 40 mTorr pressure using VirTis Co. (Gardiner, New York).

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The freeze-dried lipote fruit pulp and peel were thoroughly ground and filtered into a fine powder using an 80-mesh US standard sieve and stored in metallized bags at -20 °C until further use. On the day of experimentation, control vehicles of distilled water and LFE at varying concentrations (55, 175, 550, 2000, and 5000 mg/kg BW) were prepared by reconstituting the powder in distilled water followed by vortex mixing for one minute. The preparation and coding of LFE was done by an independent research staff from the Institute of Human Nutrition and Food (IHNF), College of Human Ecology (CHE), UPLB who was not directly involved in the conduct of oral toxicity experiment, as well as, in the data analysis to prevent any form of research bias.

Experimental animals

Ten (10) female and ten (10) male 6-week-old ICR mice were purchased from the Laboratory Animal Facility of the Research Institute for Tropical Medicine (RITM), Department of Health, Muntinlupa City, Philippines. Mice were housed individually in commercial polycarbonate cages with stainless steel tops and maintained at 22°C (±2°C) temperature, 30-60% humidity, and 12-hour:12-hour light-dark period (lights on at 7:00 A.M. and lights off at 7:00 P.M.) in the laboratory animal room of the Department of Basic Veterinary Sciences (DBVS), College of Veterinary Medicine, UPLB. Commercial maintenance mouse pellets (Altromin, Germany) and distilled water were provided in *ad libitum* basis. Mice underwent one-week acclimatization prior to experimentation.

Acute oral toxicity testing

The acute oral toxicity up-and-down-method was performed following the OECD Guideline 425 (OECD, 2008). Using the constant dose progression factor of 3.2 with 55 mg/kg BW LFE set as an initial dose, mice were randomly allocated into five (5) treatment groups (n=1 per group for both sexes) as follows: Group 1 was given 55 mg/kg BW LFE, Group 2 was given 175 mg/kg BW LFE, Group 3 was given 550 mg/kg BW LFE, Group 4 was given 2000 mg/kg BW LFE, and Group 5 was given 5000 mg/kg BW LFE. A corresponding control group given distilled water was assigned for each treatment group, hence, a total of 5 control animals. Each treatment was instituted successively from low to high dose following a 48-hour interval period to confirm the absence of mortality. Mice were fasted and weighed prior to gavaging of treatments at Day 1 of experimentation using a 1-inch 22G stainless steel gavage needle (Thermoscientific, USA) and 1 ml sterile disposable syringe (Terumo, Japan). All procedures in mice were approved by the UPLB Institutional Animal Care and Use Committee (UPLB IACUC) with an assigned approval number, CHE-2019-002.

Clinical sign of toxicity

Observation for clinical signs of toxicity was done per individual animal from each treatment group 30 minutes after vehicle and LFE administration, at the 24th hour, and daily thereafter until the 14th day of experimentation. Any abnormal changes in the fur and skin, mucous membranes and eyes, respiratory, circulatory, and nervous systems, as well as deviation from normal behavioral pattern and somatomotor activity, were recorded. Moreover, clinical signs of diarrhea, salivation, convulsions, tremors, lethargy, sleep, and coma were documented and given special attention. Clinical signs of morbidity and occurrence of mortality for all treatment groups were also accounted.

Physiologic parameters

The body weight of each mouse per treatment group was measured weekly, on Days 1, 7, and 14, using a digital top loading balance (Shimadzu, Japan) prior to administration of vehicle or LFE. On the other hand, feed and water consumptions were monitored daily. Pre-weighed feeds were given to each mouse and leftovers were collected and weighed using a digital top loading balance (Shimadzu, Japan) to compute for the daily feed intake. Likewise, a pre-determined volume of distilled drinking water was placed in properly labeled polycarbonate waterers of each mouse and leftovers were measured using a graduated cylinder to compute for the daily water intake.

Hematology and blood chemistry analysis

Blood was collected on Day 1 and Day 14 of the experimentation period before administration of vehicle and LFE treatments. A drop of tetracaine (Alcaine®, Novartis, Philippines) was instilled on the right eye of the mouse and rested for two minutes prior to the collection of blood. A total of 300µl of blood was obtained from the retro-orbital vein using a heparinized capillary tube (INRI, Netherlands) and placed into a 0.5 mL microcentrifuge tube. Ten (10) microliters of blood were used to quantify the total RBC count (tRBC), total WBC count (tWBC), and differential WBC count (dWBC) using an automated hematology analyzer (Orphée, Switzerland) whereas 250µl of blood were used to quantify the levels of kidney-filtered substances, creatinine and blood urea nitrogen (BUN) using an automated blood chemistry analyzer (Arkray Inc., Japan).

Weighing, processing, macroscopic and microscopic evaluation of tissues and organs

After the 14-day experimentation period, mice were euthanized *via* intraperitoneal injection of 60 mg/kg sodium pentobarbital (Dolethal®, UK). The thoraco-abdominal area was cut open through a midventral incision and the visceral organs including the heart, lungs, esophagus, stomach, small and large intestines, liver, spleen, and kidneys were exteriorized. The head, on the other hand, was decapitated and then cut open to collect the brain. All harvested tissues were grossly examined for any abnormalities, flushed with 0.9% sodium chloride solution, dabbed onto a clean paper towel, and weighed using a digital top loading balance. The tissues were immediately fixed in 10% buffered formalin solution for at least 72 hours, trimmed into 1 cm^3 thickness, processed using the routine paraffin technique, and sectioned at 4µm in thickness using a rotary microtome. For each organ, one out of every four sections were stained with hematoxylin and eosin (H&E) stain and examined under light microscopy (Zeiss Primostar 3, Germany). Histopathological examination was carried out by a Veterinary Pathologist who is completely blinded of the treatment assignment. The presence/absence and the magnitude of the following lesions were ascertained: cellular, inflammatory, proliferative, and degenerative responses, neoplasia, and healing processes.

Statistical analysis

Results for the mean body weight and mean daily feed and water intakes were analyzed using Independent Sample T-Test. All analyses were performed using SPSS v.23 (IBM Corp., Armonk, NY, USA) and significant differences were determined at $p<0.05$.

RESULTS

Effect of LFE on morbidity and mortality and clinical signs of toxicity

Throughout the experimental period, no treatment-related morbidities and mortalities were recorded in both sexes of ICR mice receiving distilled water or increasing concentrations of LFE. In particular, no abnormal changes in the physical appearance, behavior, neurologic, circulatory or respiratory signs were noted (Tables 1 and 2).

Effect of LFE on body weight

Administration of LFE did not adversely affect the mean body weight and mean body weight gain of male and female ICR mice showing

Table 1: Effect of varying doses of LFE on the physical, behavioral, neurologic, circulatory and respiratory signs of male ICR mice.

Parameters	30 minutes post-administration						24 hours					
	Control	55 mg/kg LFE	175 mg/kg LFE	550 mg/kg LFE	2000 mg/kg LFE	5000 mg/kg LFE	Control	55 mg/kg LFE	175 mg/kg LFE	550 mg/kg LFE	2000 mg/kg LFE	5000 mg/kg LFE
Abnormal skin	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal fur	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal eyes	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal mucous mem.	A	A	A	A	A	A	A	A	A	A	A	A
Tremor	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A	A	A	A	A
Salivation	A	A	A	A	A	A	A	A	A	A	A	A
Diarrhea	A	A	A	A	A	A	A	A	A	A	A	A
Lethargy	A	A	A	A	A	A	A	A	A	A	A	A
Sleep	A	A	A	A	A	A	A	A	A	A	A	A
Coma	A	A	A	A	A	A	A	A	A	A	A	A
Parameters	**7 days**						**14 days**					
	Control	55 mg/kg LFE	175 mg/kg LFE	550 mg/kg LFE	2000 mg/kg LFE	5000 mg/kg LFE	Control	55 mg/kg LFE	175 mg/kg LFE	550 mg/kg LFE	2000 mg/kg LFE	5000 mg/kg LFE
Abnormal skin	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal fur	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal eyes	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal mucous mem.	A	A	A	A	A	A	A	A	A	A	A	A
Tremor	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A	A	A	A	A
Salivation	A	A	A	A	A	A	A	A	A	A	A	A
Diarrhea	A	A	A	A	A	A	A	A	A	A	A	A
Lethargy	A	A	A	A	A	A	A	A	A	A	A	A
Sleep	A	A	A	A	A	A	A	A	A	A	A	A
Coma	A	A	A	A	A	A	A	A	A	A	A	A

Table 2: Effect of varying doses of LFE on the physical, behavioral, neurologic, circulatory and respiratory signs of female ICR mice.

Parameters	30 minutes post-administration						24 hours					
	Control	55 mg/kg LFE	175 mg/kg LFE	550 mg/kg LFE	2000 mg/kg LFE	5000 mg/kg LFE	Control	55 mg/kg LFE	175 mg/kg LFE	550 mg/kg LFE	2000 mg/kg LFE	5000 mg/kg LFE
Abnormal skin	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal fur	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal eyes	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal mucous mem.	A	A	A	A	A	A	A	A	A	A	A	A
Tremor	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A	A	A	A	A
Salivation	A	A	A	A	A	A	A	A	A	A	A	A
Diarrhea	A	A	A	A	A	A	A	A	A	A	A	A
Lethargy	A	A	A	A	A	A	A	A	A	A	A	A
Sleep	A	A	A	A	A	A	A	A	A	A	A	A
Coma	A	A	A	A	A	A	A	A	A	A	A	A
Parameters	**7 days**						**14 days**					
	Control	55 mg/kg LFE	175 mg/kg LFE	550 mg/kg LFE	2000 mg/kg LFE	5000 mg/kg LFE	Control	55 mg/kg LFE	175 mg/kg LFE	550 mg/kg LFE	2000 mg/kg LFE	5000 mg/kg LFE
Abnormal skin	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal fur	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal eyes	A	A	A	A	A	A	A	A	A	A	A	A
Abnormal mucous mem.	A	A	A	A	A	A	A	A	A	A	A	A
Tremor	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A	A	A	A	A
Salivation	A	A	A	A	A	A	A	A	A	A	A	A
Diarrhea	A	A	A	A	A	A	A	A	A	A	A	A
Lethargy	A	A	A	A	A	A	A	A	A	A	A	A
Sleep	A	A	A	A	A	A	A	A	A	A	A	A
Coma	A	A	A	A	A	A	A	A	A	A	A	A

results that were comparable to the control mice of the same group. Although a 0.07g reduction in the mean body weight gain was observed in the female mouse receiving 2000 mg/kg dose LFE, this decrement is virtually negligible as female mouse treated with the highest dose of 5000 mg/kg LFE posted a positive mean body weight gain value of 0.23 g (Figure 1).

Effect of LFE on feed and water intake

Similar to their control counterparts, male and female mice administered with increasing concentrations of LFE had normal to high mean daily feed intake (Figure 2). As shown in Figure 3, both distilled water- and LFE-treated mice, irrespective of the treatment group and sex, consumed a considerably greater volume of water than the established normal average daily water intake for ICR mice.

Effect of LFE on hematology

The baseline and endline tRBC, tWBC, and dWBC values of male ICR mice supplemented with varying concentrations of LFE fell within the normal range of their control counterparts with the exception of the 2000 mg/kg dose LFE mouse, which displayed a minimal reduction in the tRBC (3.70x10^6/µl), tWBC (2.00 x10^3/µl), and lymphocytes values (1.80 x10^3/µl) at the 14th day of the experimental period (Figure 4). In the case of female mice, all animals belonging to the control and LFE treatment groups presented a baseline and endline tRBC, tWBC, and dWBC that rest within the normal limits for ICR mice except for a slight increase in the baseline monocyte count of control (1.50 x10^3/µl) and treatment mice given 2000 mg/kg LFE (1.50 x10^3/µl), as well as mouse treated with 5000 mg/kg LFE (1.50 x10^3/µl). These values, however, ultimately returned to an acceptable range at the 14th day of the experimental period (Figure 5).

Effect of LFE on creatinine and blood urea nitrogen levels

Irrespective of the sex, mice belonging to various LFE treatment groups exhibited normal creatinine levels which closely approximated those of their corresponding control groups (Figure 6). On the other hand, the

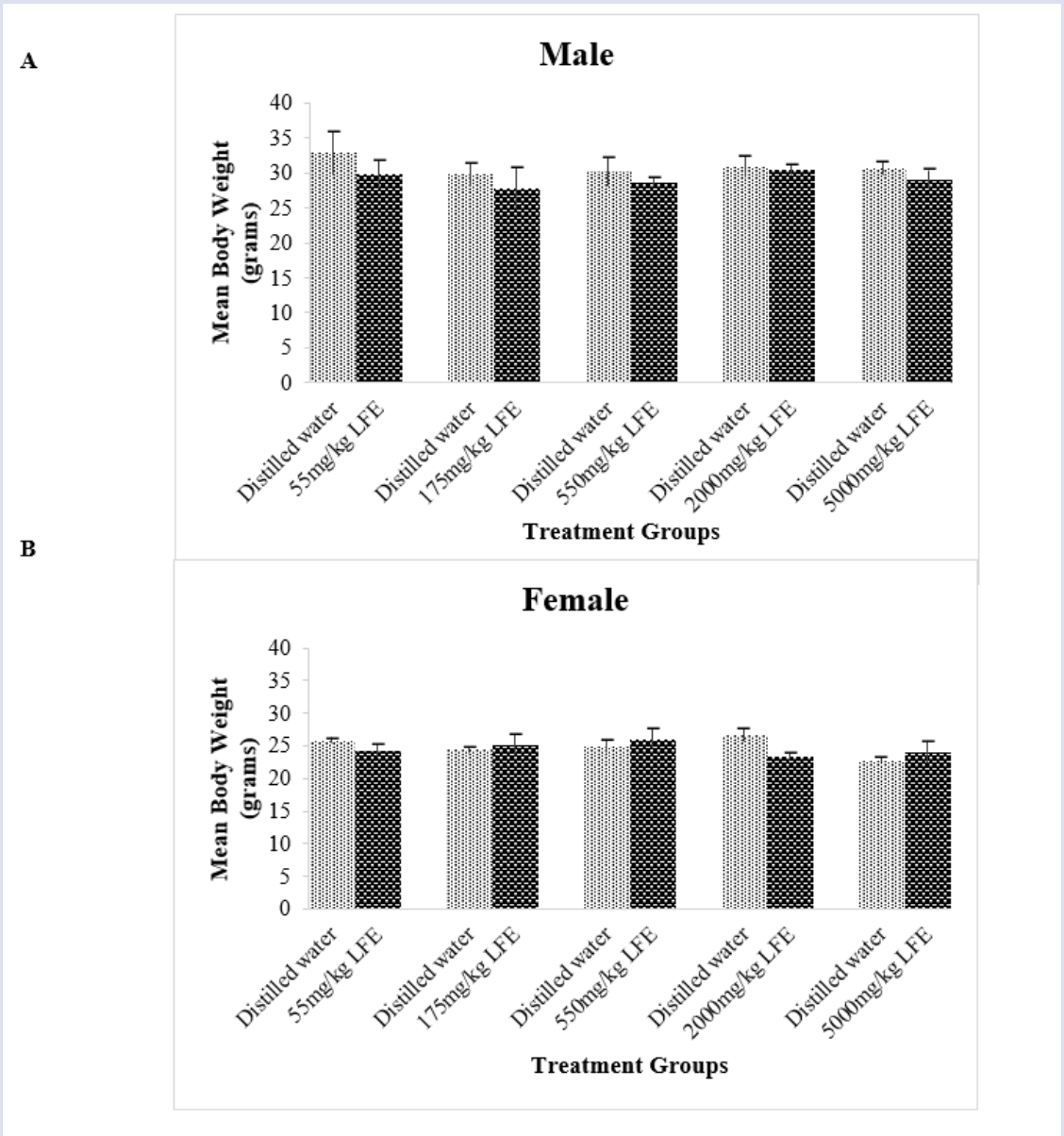

Figure 1: Mean body weight of male (A) and female (B) mice given distilled water and varying doses of lipote fruit extract (LFE) (at column width).

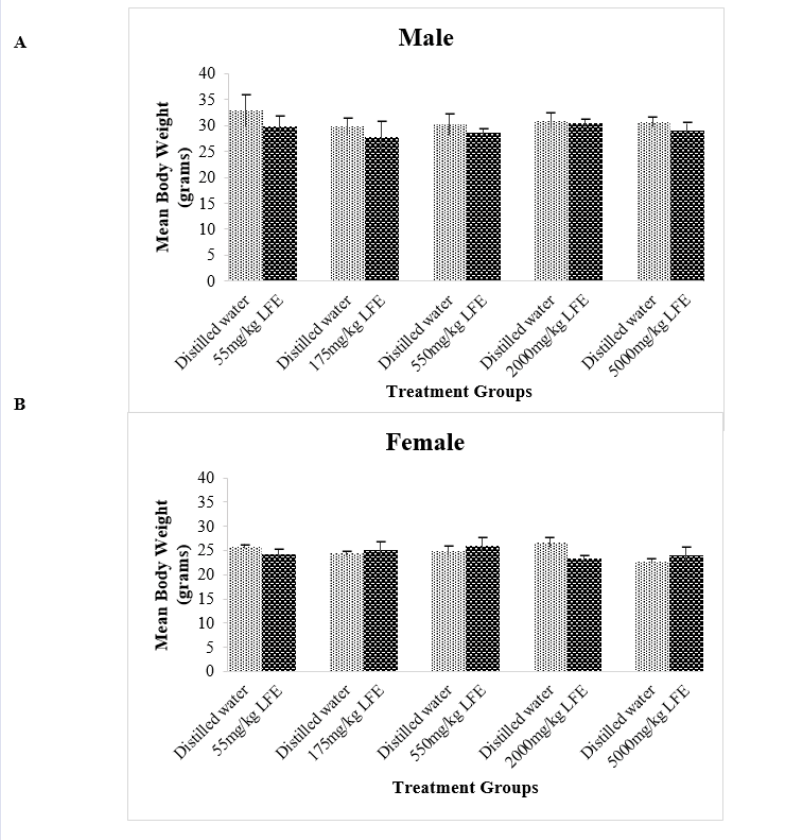

Figure 2: Mean daily feed intake of male (A) and female (B) ICR mice given distilled water and varying doses of lipote fruit extract (LFE). (at column width).

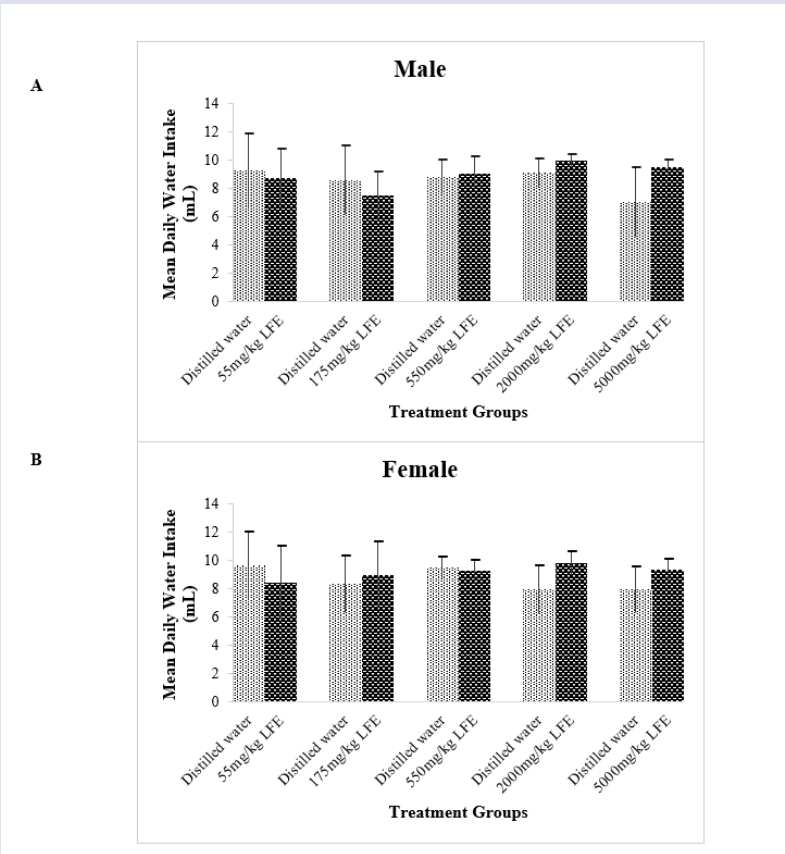

Figure 3: Mean daily water intake of male (A) and female (B) ICR mice given distilled water and varying doses of lipote fruit extract (LFE). (at column width).

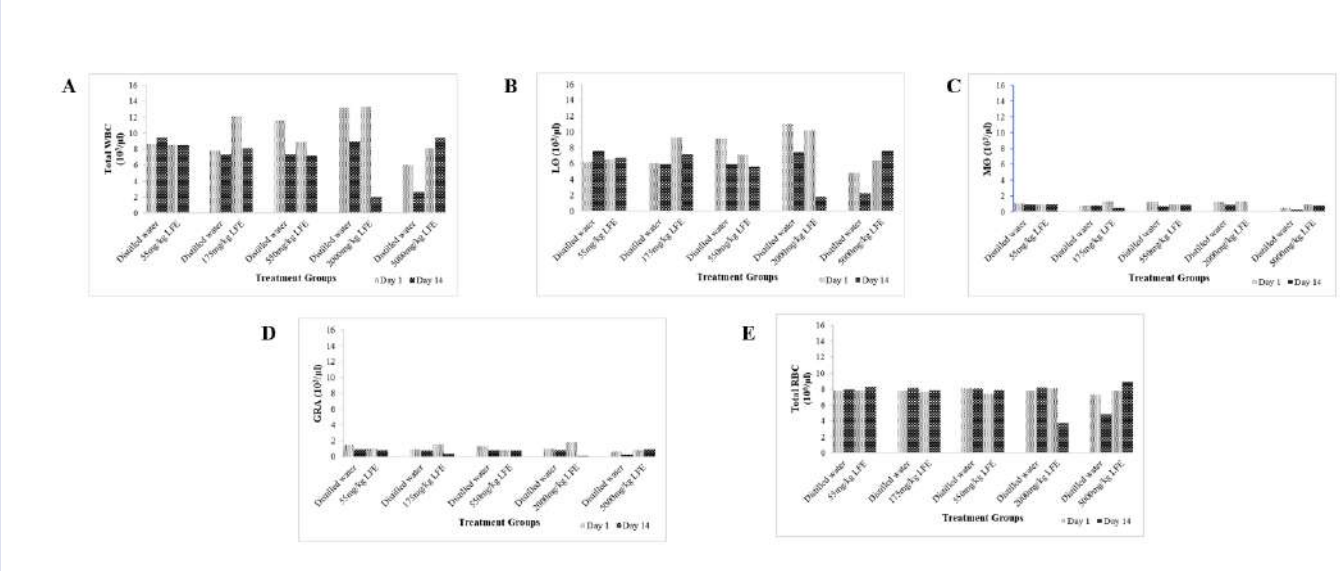

Figure 4: Hematological profile of male ICR mice given distilled water and varying doses of lipote fruit extract (LFE). Legend: A – total white blood cell (WBC) count (10^3/µl), B – lymphocyte (LYM) count (10^3/µl), C – monocyte (MON) count (10^3/µl), D - granulocyte (GRA) count (10^3/µl), E – total red blood cell (RBC) count (10^6/µl). (at column width).

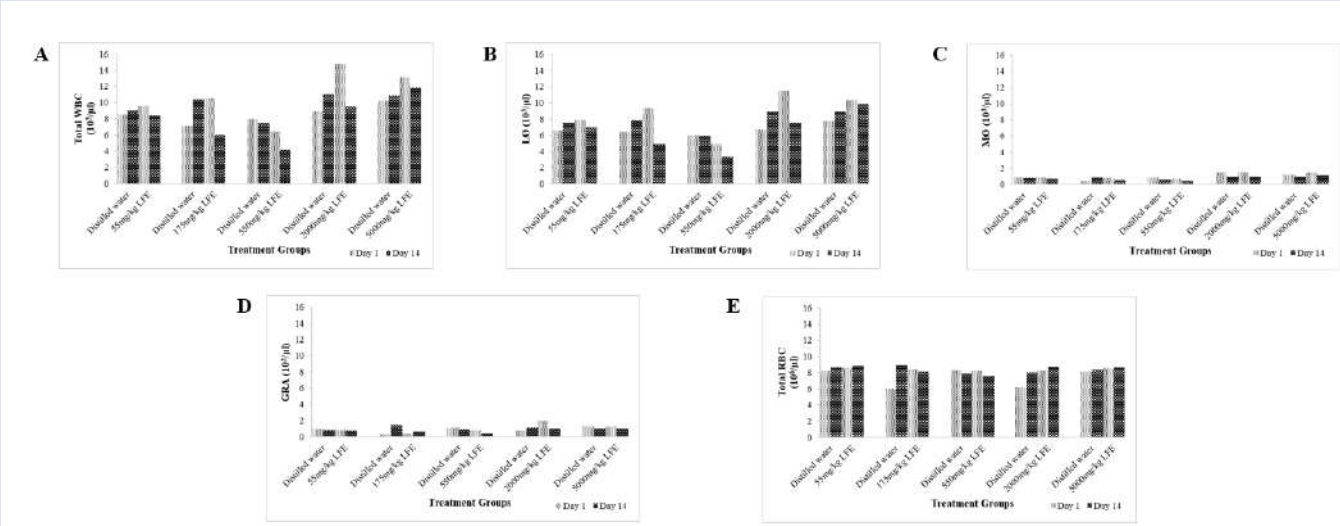

Figure 5: Hematological profile of female ICR mice given distilled water and varying doses of lipote fruit extract (LFE). Legend: A – total white blood cell (WBC) count (10^3/µl), B – lymphocyte (LYM) count (10^3/µl), C – monocyte (MON) count (10^3/µl), D - granulocyte (GRA) count (10^3/µl), E – total red blood cell (RBC) count (10^6/µl). (at column width).

BUN levels across all treatment groups fell within the normal range for both sexes with the exception of the male (both control and treated) and female mouse given 2000 mg/kg dose LFE whose BUN levels were slightly decreased at day 1 but eventually returned to normal values upon reaching day 14 of the experimental period (Figure 7).

Effect of LFE on relative organ weights, macroscopic, and microscopic appearance of harvested organs

Following sacrifice, the gross features of the harvested organs from both male (Figures 8) and female (Figures 9) mice treated with varying concentrations of LFE closely resembled those of their vehicle controls revealing absence of any significant gross morphological alterations as evidenced by the apparently normal color, texture, shape and size of the tissues. This was further confirmed by the findings of comparable organs weights between vehicle and LFE treatment groups except for a slightly higher values obtained for the stomach in male and female mice given 175 mg/kg LFE, which can be possibly ascribed to the incomplete evacuation of its contents (Figures 10 and 11). At the microscopic level, all prepared tissue sections irrespective of the treatment group presented a normal histological architecture that was devoid of any discernible pathological changes such as necrosis, immune cell infiltration, neoplasia, hemorrhages and signs of degenerative and proliferative responses. For example, the organs of the gastrointestinal tract such as the stomach, small intestines and large intestines displayed a well preserved mucosal epithelium that is lined by numerous secretory and/or absorptive cells; the lungs presented an intact alveolar structures; the kidneys showed normal appearance of the renal tubules and nephrons; and the liver exhibited a properly oriented hepatic parenchyma containing radially arranged hepatocytes that are separated by sinusoids (Figure 12).

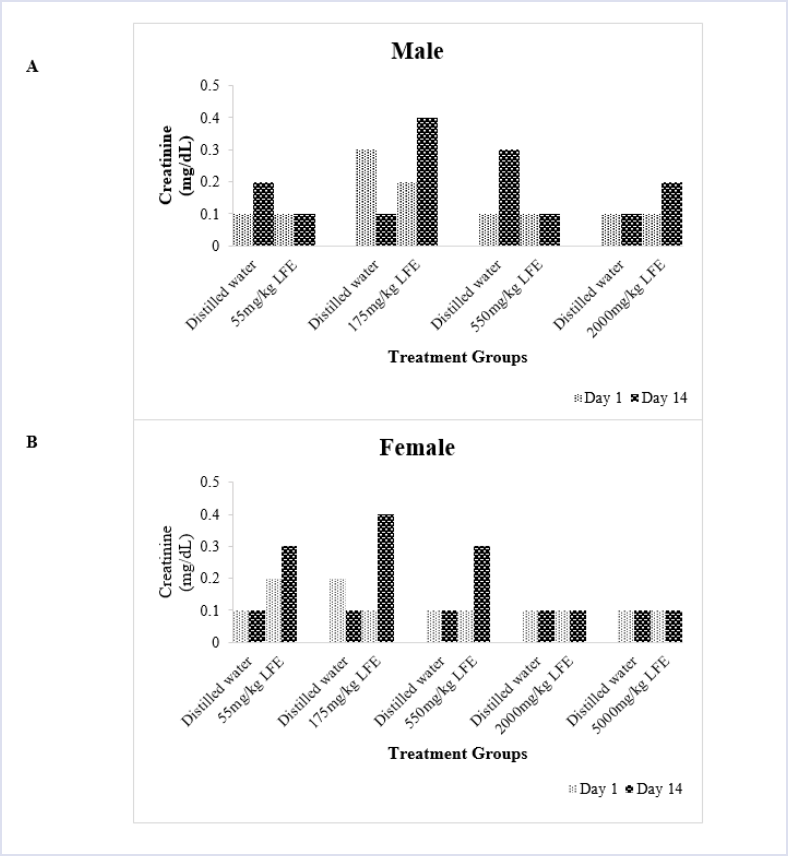

Figure 6: Blood creatinine of male (A) and female (B) ICR mice given distilled water and varying doses of lipote fruit extract (LFE). (at column width).

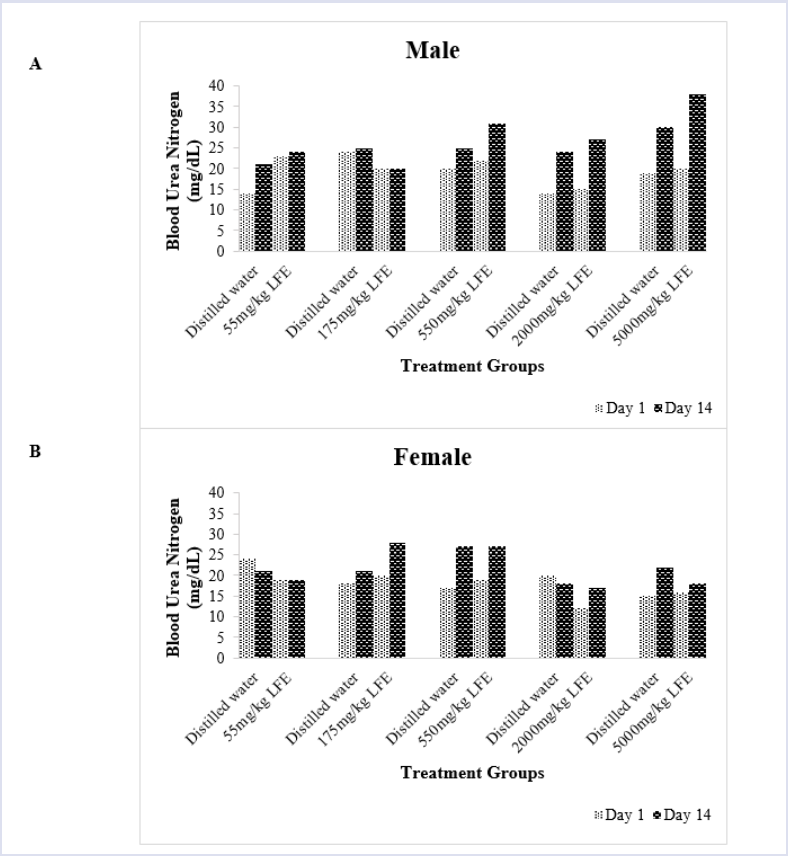

Figure 7: Blood urea nitrogen of male (A) and female (B) ICR mice given distilled water and varying doses of lipote fruit extract (LFE). (at column width).

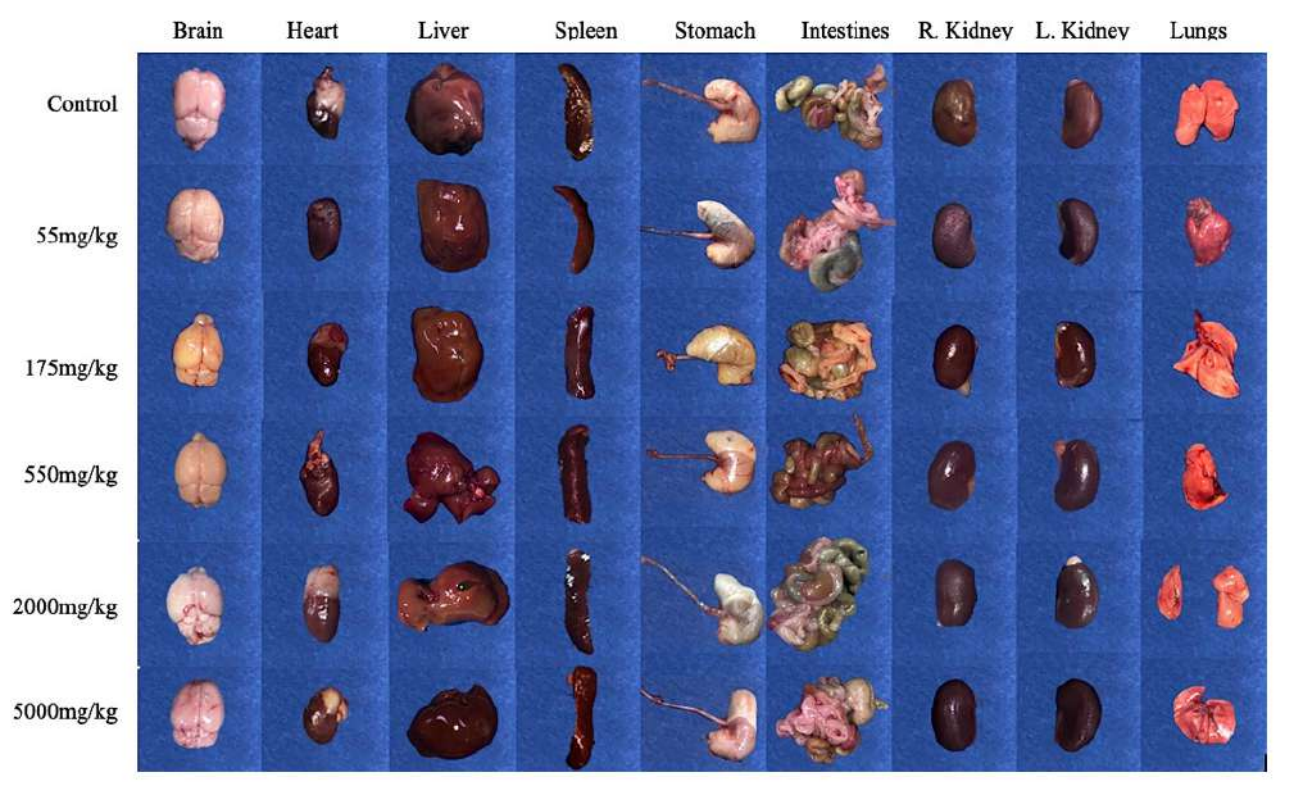

Figure 8: Gross morphology of selected organs in male ICR mice given with distilled water (control group) and varying concentrations of freeze-dried lipote fruit extract (LFE) (treatment group). (at full page width).

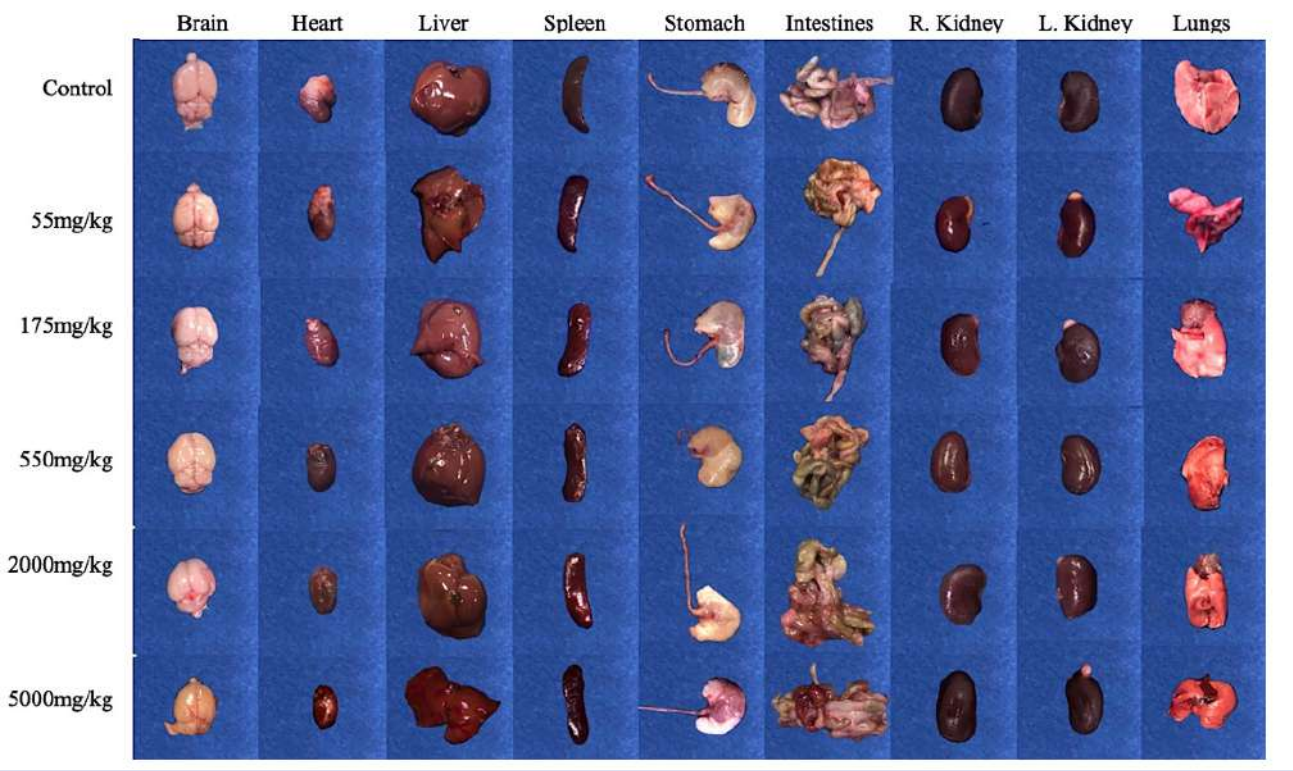

Figure 9: Gross morphology of selected organs in female ICR mice given with distilled water (control group) and varying concentrations of freeze-dried lipote fruit extract (LFE) (treatment group) (at full page width).

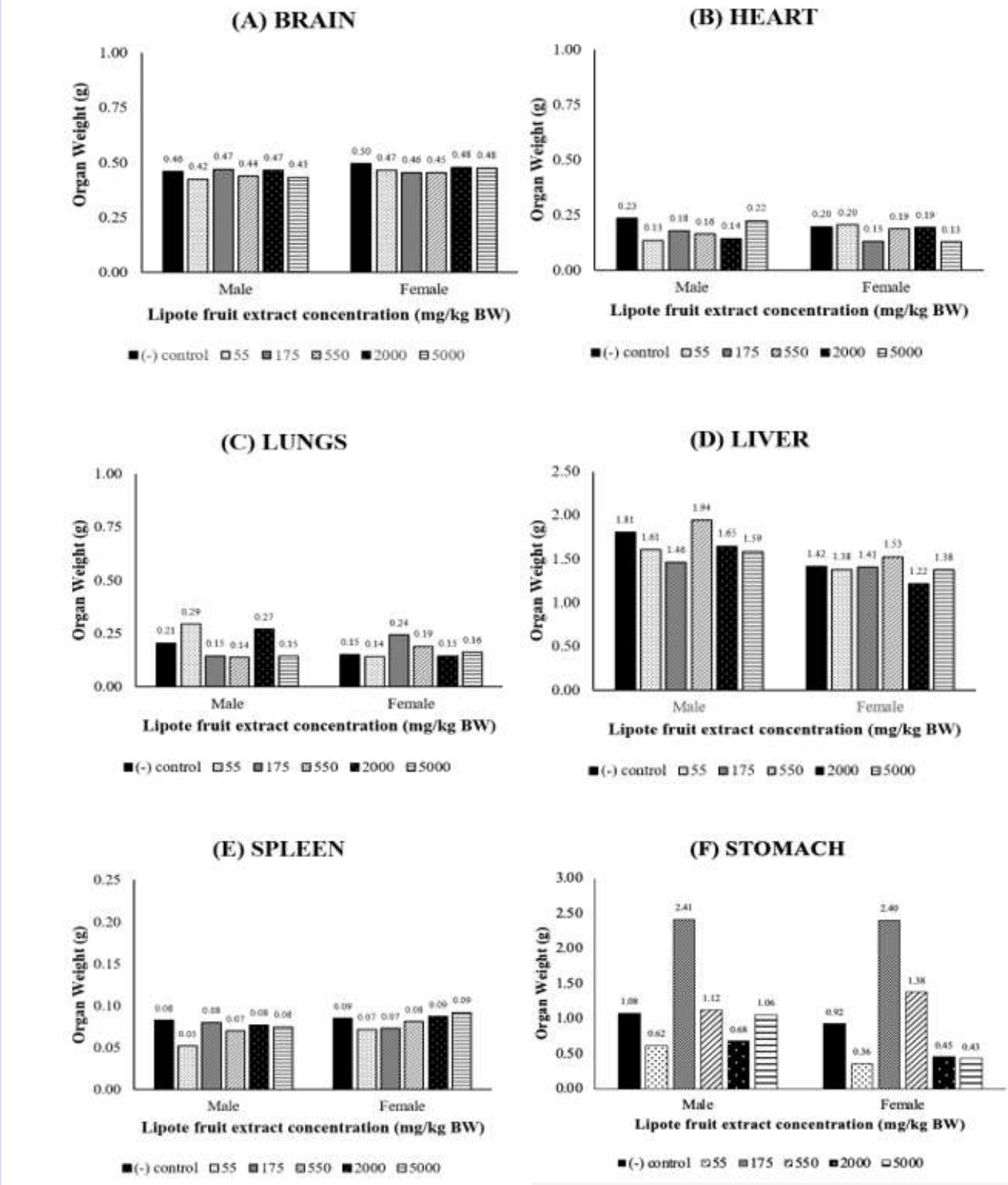

Figure 10: Organ weights of the (A) brain, (B) heart, (C) lungs, (D) liver, (E) spleen, (F) stomach in male and female ICR mice given with distilled water (control group) and varying concentrations of freeze-dried lipote fruit extract (LFE) (treatment group). (at full page width).

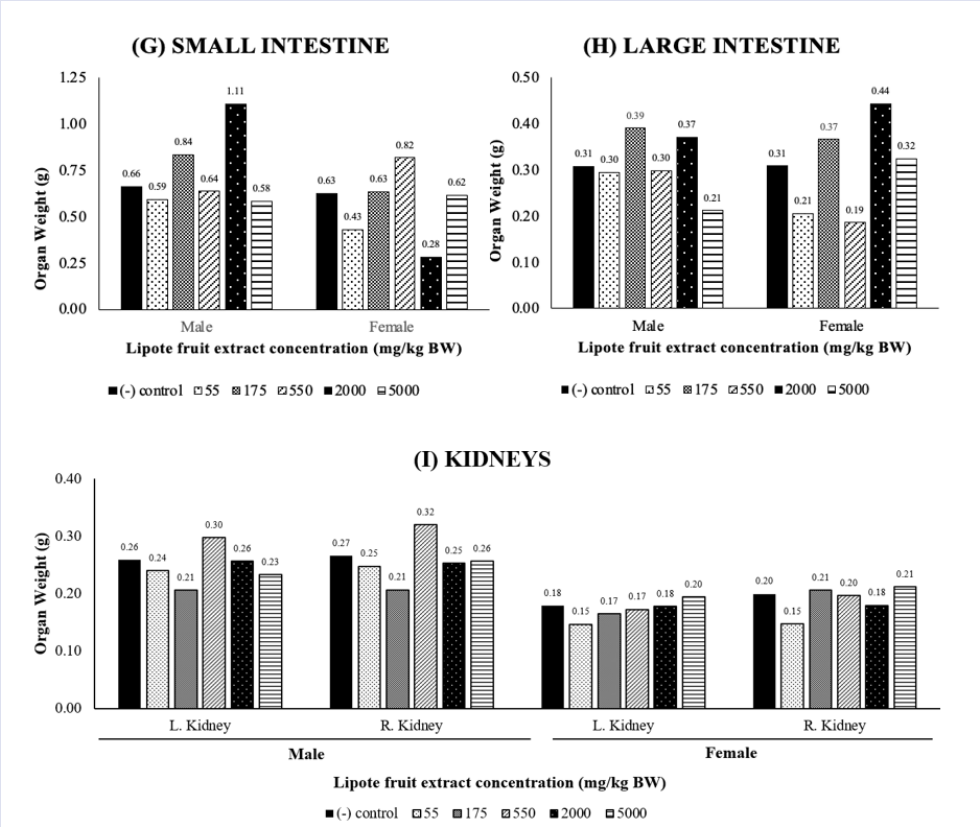

Figure 11: Organ weights of the (G) small intestine, (H) large intestine, and (I) left and right kidneys in male and female ICR mice given with distilled water (control group) and varying concentrations of freeze-dried lipote fruit extract (LFE) (treatment group). (at full page width).

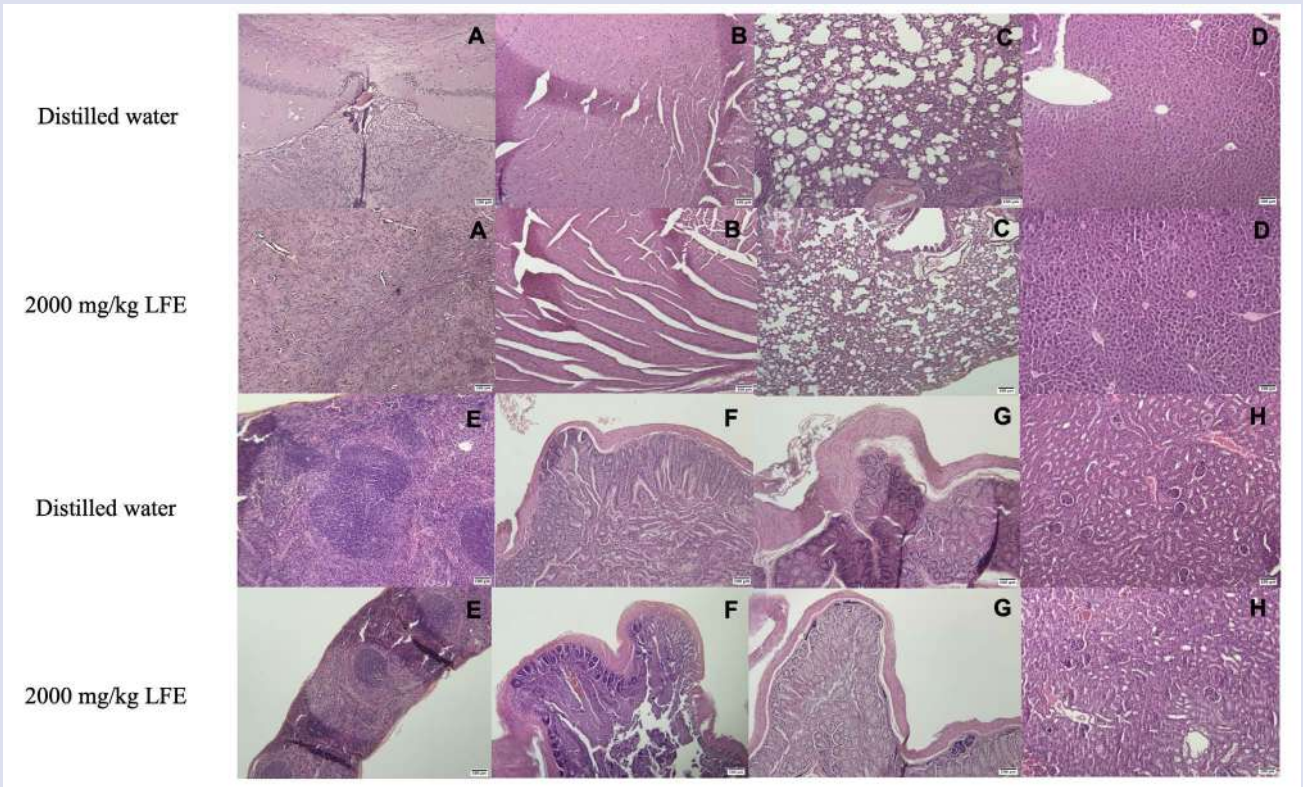

Figure 12: Representative sections of the histological findings of the brain (A), heart (B), lungs (C), liver (D), spleen (E), small intestine (F), large intestine (G), and kidney (H) of ICR mice given the control (distilled water) and lipote fruit extract (LFE) (2000 mg/kg BW). Hematoxylin and eosin (H&E, x40) stain. Scale bar: 100μm. (at full page width).

DISCUSSION

Like other plants, berries under the Genus *Syzygium* have been culturally and socially accepted as a mode of medicinal therapy or as a form of indigenous remedy especially for low-income communities to address various human conditions such as diabetes, dysentery, hypertension, cough and high blood pressure. This has prompted a growing number of scientific investigations that evaluated the safety of *Syzygium* species including *S. cumini*,[16,17] *S. guineense*,[18,19] *S. kanarense*,[20] *S. polyanthum*,[21] and *S. aromaticum*.[22] However, no toxicity studies have been documented to date which specifically establish the safety profile of *S. polycephaloides* or lipote. Therefore, an acute oral toxicity test was undertaken to determine the possible detrimental effects of freeze-dried LFE on various physiologic, clinico-behavioral, hematological, biochemical, macroscopic, biometric and microscopic parameters using the OECD Guideline 425.

Administration of increasing doses of LFE in male and female ICR mice did not produce any treatment-related mortality throughout the duration of the experimentation period. In addition, LFE failed to elicit any obvious signs of morbidity as no discernible changes in the physical, behavioral, neurologic, circulatory, and cardiorespiratory parameters were detected. Similar to our findings, it was reported in previous studies that no observable signs of toxicity were noted in both sexes of Swiss mice given *S. cumini* extracts with concentrations ranging from 50 mg/kg BW up to as high as 10,125 mg/kg BW.[17,23] However, instead of freeze-dried fruit extracts, these studies preferentially utilized an ethanolic stem bark extracts and hydroalcoholic leaf extracts, respectively. Meanwhile, a maximum dose of 2000 mg/kg BW was found to be well tolerated by *S. cumini* seed extract-treated male and female Wistar rats,[24] *S. kanarense* leaf extract-treated female Wistar Albino rats,[20] and *S. aqueum* leaf extract-treated male Sprague-Dawley rats.[25] Additionally, *S. guineense* leaf methanolic extract-treated female Wistar rats exhibited zero toxicity signs and mortality at doses as high as 5000 mg/kg BW.[19] Taken together, our results suggest that LFE is relatively innocuous and that this property appears to be shared by closely related species of *Syzygium*.

Consistent with these clinico-behavioral aspects, the physiological parameters of male and female mice across various LFE treatment groups closely matched their corresponding controls and fell within the normal limits set for the species. In particular, the baseline and endline mean body weight of vehicle and LFE-treated mice were found to be within the established normal range of 20-40 g for adult mice.[26] Although, a 0.07 g reduction in the mean body weight gain was posted by the female mouse receiving 2000 mg/kg LFE, this inconsistency is rather insignificant since the female mouse treated with the maximum dose of 5000 mg/kg BW LFE even exhibited a positive mean value of 0.23 g. Additionally, all treatment groups including the mouse given 2000 mg/kg BW LFE recorded a mean daily feed and water intake which fell within the published average normal range of 12-18 g/100 g BW/day and 15 ml/100 g BW/day, respectively.[26] In consonance with these results, there were no significant differences in the BW and feed and water intake of male and female Swiss mice and Wistar rats given hydroalcoholic leaf extracts of *S. cumini* at increasing concentrations of 0.05, 0.1, and 0.25 g/kg BW[17] while no deleterious effect on these physiological parameters was perceived using Sprague-Dawley rats administered with a single dose of 2,000 mg/kg BW *S. aqueum* leaf extracts.[25] Furthermore, normal body weight in 2000 and 5000 mg/kg BW *S. guineense* leaf methanolic extract-treated male and female Wistar rats and 2000 mg/kg BW aqueous *S. kanarense* leaf extract-treated female Wistar rats, respectively, have been previously recorded.[19-20] Collectively, the present findings lend further justification that LFE does not seem to cause any potential adverse health effects.

Hematological parameters such tRBC, tWBC, and dWBC counts of LFE-treated mice, irrespective of sex, resembled those of their control counterparts and remained within the acceptable range values for ICR mice.[26,27] The sole exception was the male mouse given 2000 mg/kg dose LFE, which demonstrated a slightly lowered tRBC, tWBC, and lymphocyte count at day 14 of the experimental period. This observed decrement in tRBC could be explained by the increased degree of physical activity of the animal prior to blood collection as exhausted mouse tend to show lower red blood cell count as compared to sedentary ones.[28] On the other hand, the decreased in the total circulating levels of leukocytes and lymphocyte count may be triggered by stress due to handling and technical manipulations.[29,30] However, owing to the fact that this particular animal did not manifest any abnormal changes on its physiological and clinico-behavioral parameters and that mouse administrated with the highest dose of 5000 mg/kg LFE had normal hematological profiles, it can be inferred that the identified disparity in the hematological profile is probably not treatment-related. For the female mice, the slight elevation of the baseline monocyte counts in mice treated with 2000 mg/kg LFE and 5000 mg/kg LFE may also be brought about by acute stress due to restraint[31] since the values eventually returned to normal levels at day 14 of the experimentation period. Altogether, these findings, along with previous reports that hematological data remained unaffected after oral supplementation with extracts derived from related species of *Syzygium* as in the case of *S. kanarense*,[20] *S. aromaticum*,[22] and *S. cumini*,[17] further support the notion about the pronounced safety of LFE.

The circulating blood levels of creatinine and BUN were subsequently examined. Both sexes of ICR mice treated with varying concentrations of LFE displayed blood levels of both these kidney-filtered substances that markedly approximated those of their control counterparts and that rest within the normal reference values as those in some previous studies.[26,27] The slight reduction of the baseline BUN levels attained in both 2000 mg/kg LFE-treated male and female mice may be attributed to a lower dietary protein intake during the acclimatization period.[32] In agreement with our data, the levels of BUN and creatinine were not significantly altered in Swiss mice and Wistar rats treated with hydroalcoholic extracts of *S. cumini* leaf extract,[17] Sprague-Dawley rats treated with *S. aqueum* leaf extracts,[25] and Wistar Albino rats treated with *S. aromaticum* ethanolic fruit extract,[22] and *S. kanarense* ethyl acetate leaf extracts.[20] On the basis of these accounts, it may be inferred that LFE profoundly preserves the integrity of the kidney.

Finally, assessment of the gross and microscopic parameters, and organs weights of harvested tissue samples of the stomach, liver, spleen, kidneys, lungs, heart and brain in various LFE treatment groups disclosed no evidence of considerable morphological abnormalities. These observations are in parallel with their respective controls and in previous studies involving the use of related *Syzygium* species such as *S. guineense*,[18,19] *S. aromaticum*,[22] *S. karanense*,[20] *S. aqueum*,[25] and *S. cumini*.[17] Together, these findings further reinforced the apparent safety of LFE.

CONCLUSION

Overall, the findings of the present study suggest that freeze-dried LFE is non-toxic, relatively safe for consumption, and has an LD_{50} above 5000 mg/kg. It is recommended that sub-chronic and chronic toxicity studies be done to determine the safety of continuous oral ingestion of LFE.

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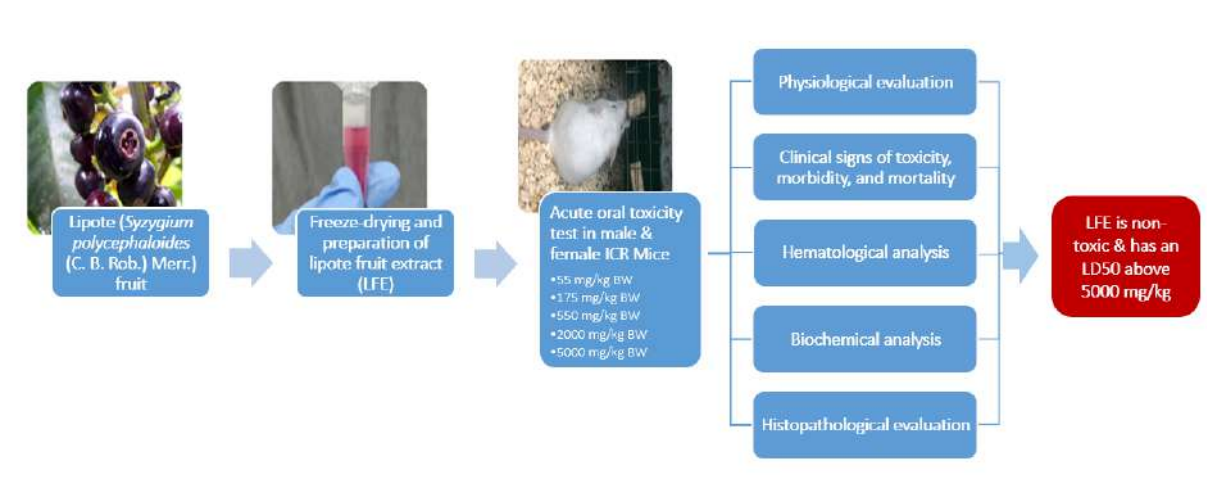
REFERENCES

1. Florido HB, Cortiguerra FF. Lesser Known Edible Tree Species. Research Information Series on Ecosystems. Laguna: Department of Environment and Natural Resources. Ecosystems Res Development Bureau. 2003;15(3):1-8.
2. Taguiling NK. Macrofloral biodiversity conservation in Ifugao. Eur Sci J. 2013;4:469-82.
3. Guiam V. Lipote: The munchy bunch. BAR Res Development Digest. 2012;14:7-8.
4. Jose AS, De Guzman GB. Lipote: *Syzigium polycephaloides* (C.B. Rob.) Merr. Research Information Series on Ecosystems. Laguna: Department of Environment and Natural Resources. Ecosystems Res Development Bureau. 2017;29(2):1-10.
5. Santiago DM, Garcia VV, Dizon EI, Merca FI. Antioxidant activities, flavonol and flavanol content of selected Southeast Asian indigenous fruits. Philipp Agric Sci. 2007;90(2):123-30.
6. Ragasa CY, Torres OB, Shen CC, Lahica MK, Sulit AB, Chua DB, *et al.* Triterpentenes from the leaves of *Syzygium polycephalum*, *S. cumini*, and *S. samarangense*. Chem Nat Compd. 2014;50:942-4.
7. Indrawati I, Rossiana N, Hidayat T. Antibacterial Activity of Bacterial Endophytes from Kupa Plant (*Syzygium Polycephalum* Miq. (Merr & Perry) Against Pathogenic Bacteria. IOP Conference Series: Earth Env Sci. 2018;166(1):012013.
8. Jemi R, Syafii W, Febrianto F, Hanafi M. Antifungal Properties of Kupa Wood (*Syzygium polycephalum* Mig.). J Trop For Sci. 2010;8(2):93-108.
9. Hartanti L, Yonas SMK, Mustamu JJ, Wijaya S, Setiawan HK, Soegianto L. Influence of extraction methods of bay leaves (*Syzygium polyanthum*) on antioxidant and HMG-CoA Reductase inhibitory activity. Heliyon. 2019;5(4):e01485.
10. Nigam V, Nigam R, Singh A. Distribution and medicinal properties of *Syzygium* species. Cur Res Pharm. Sci. 2012;2:73-80.
11. Juanda D, Aligita W, Elfahmi, Hartati R, Musaad S. Antioxidant and alpha glucosidase inhibition activity of kupa (*Syzygium polychepalum* miq.) cortex. Int J Pharm Phytopharmacological Res. 2018;8(3):33-8.
12. Novotny L, Vachalkova A, Biggs D. Ursolic acid: An anti-tumorigenic and chemopreventive activity. Neoplasma. 2001;48(4):241-46.
13. Zhou R, Zhang Z, Zhao L, Jia C, Xu S, Mai Q, *et al.* Inhibition of mTOR signaling by oleanolic acid contributes to its anti-tumor activity in osteosarcoma cells. J Orthop Res. 2011;29(6):846-52.
14. Nishizawa M, Yamada H, Sano J, Ito S, Hayashi Y, Ikeda H, *et al.* Structure of syzygiol: A skin tumor promotion inhibitor. Tetrahedron Lett. 1991;2:211-2.
15. Organization for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals 425: Acute Oral Toxicity - Up-and-down Procedure. Paris: OECD Publishing. 2008;1-26.
16. Ugbabe GE, Ezeunala MN, Edmond I, Apev J, Salawu OT. Preliminary Phytochemical, Antimicrobial and Acute Toxicity Studies of the Stem, bark and the Leaves of a cultivated *Syzygium cumini* Linn. (Family: Myrtaceae) in Nigeria. Afr J Biotechnol. 2010;9(41):6943-7.
17. Silva SD, Abreu IC, Silva GF, Ribeiro RM, Lopes AD, Cartagenes MD, *et al.* The toxicity evaluation of *Syzygium cumini* leaves in rodents. Rev Bras Farmacogn. 2012;22(1):102-8.
18. Okwuofu EO, Ozolua RI, Yibala OI, Akhigbemen AM. Evaluation of acute and sub-acute safety profile of *Syzygium guineense* (Wild) DC methanol stem bark extract in rodents. J Pharm Innov. 2017;6(6):174-9.
19. Loha M, Mulu A, Abay SM, Ergete W, Geleta B. Acute and subacute toxicity of methanol extract of Syzygium guineense leaves on the histology of the liver and kidney and biochemical compositions of blood in rats. Evid Based Complement Alternat Med. 2019;2019:1-15.
20. Divakar MS, Chalannavar RJ, Debnath S, Chatterjee TJ, Sooryaprakash SH. Evaluation of acute toxicity exposure to aqueous leaves extract of *Syzygium kanarense* (Talbot) Raizada. J Microbiol Biotechnol Food Sci. 2021;10(5):1-7.
21. Ramli S, Radu S, Shaari K, Rukayadi Y. Toxicity analysis of *Syzygium polyanthum* (Wight) Walp. leaves extract and its stability against different pH and temperature. Emir J Food Agric. 2020;11(6):461-8.
22. Saeed TA, Osman OA, Amin AE, Badwi SM. Safety assessment and potential anti-inflammatory effect of ethanolic extract of Syzygium aromaticum in albino rats. Adv Biosci Biotechnol. 2017;8(11):411-20.
23. Muruganandan S, Srinivasan K, Chandra S, Tandan CK, Lal J, Raviprakash V. Anti-inflammatory activity of *Syzygium cumini* bark. Fitoterapia. 2001;72(4):369-75.
24. Kumar A, Ilavarasan R, Jayachandran J, Deecaraman M, Kumar RM, Aravindan P, *et al.* Anti-inflammatory activity of *Syzygium cumini* seed. Afr J Biotechnol. 2008;7(8):941-3.
25. Manaharan T, Chakravarthi S, Radhakrishnan AK, Palanisamy UD. In vivo toxicity evaluation of a standardized extract of *Syzygium aqueum* leaf. Toxicol Rep. 2014;1:718-25.
26. Suckow MA, Danneman P, Brayton C. The Laboratory Mouse. Florida: CRC Press. 2001;8-21.
27. Serfilippi LM, Pallman DRS, Russell B, Spainhour CB. Serum clinical chemistry and hematology reference values in outbred stocks of albino mice from three commonly used vendors and two inbred strains of albino mice. Contemp Top Lab Anim Sci. 2003;42(3):46-52.
28. Liu Q, Wang J, Cui J, Yang Y, Du G. A novel acute anemia model for pharmacological research in mice by compelled acute exercise. Acta Pharmacol Sin. 2009;30(12):1643-7.
29. Li YF, Yuan L, Xu YK, Yang M, Zhao YM, Luo ZP. Antistress effect of oligosaccharides extracted from *Morinda officinalis* in mice and rats. Acta Pharmacol Sin. 2001;22(12):1084-8.
30. Schwab CL, Fan R, Zheng Q, Myers LP, Hebert P, Pruett SB. Modeling and predicting stress-induced immunosuppression in mice using blood parameters. Toxicol Sci. 2005;83(1):101-13.
31. Jiang W, Li Y, Sun J, Li L, Li J, Zhang C, *et al.* Spleen contributes to restraint stress induced changes in blood leukocytes distribution. Sci Rep. 2017;7(1):1-10.
32. Constable PD, Hinchcliff KW, Done SH, Grunberg W. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. 11th Ed. Missouri: Elsevier. 2016;626-8.

GRAPHICAL ABSTRACT



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