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TOXICOLOGICAL PROFILING OF LONG-TERM ORAL ADMINISTRATION OF ULTRASOUND-ASSISTED ETHANOLIC LEMONGRASS LEAVES EXTRACT IN SPRAGUE DAWLEY RATS

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ABSTRACT

The study investigated the toxicological profile of lemongrass leaf extract obtained by ultrasound-assisted ethanolic (UAE) technique in Sprague Dawley (SD) rats. Extracts of dried lemongrass leaves were obtained by the UAE technique. A single oral dose of 5000mg/kg body weight of the extract was administered by gavage to ten (10) female SD rats, and 1 mL/100g bw.t of normal saline was used as a vehicle for the control rats for acute toxicity studies. An hourly observation was made on the rats for the first 12 hours, and a 24-hour observation was made after 24, 48 hours, up to 14 days for any indications of acute toxicity. Another set of rats was also administered, respectively, with 200, 600, and 1200mg/kg bw.t doses of the extract for six weeks to determine its maximum permissible dosage and long-term toxicological effect. The SD rats› body weights were recorded. Urine and blood samples were collected for urinalysis, clinical chemistry, and haematological analysis, respectively. The heart, kidney, liver, spleen, and lungs were harvested after sacrificing the rats for histopathological examination after the termination of the experiment. At the end of the study, no mortality or morbidity was recorded for both the acute and subchronic studies nor were any signs of clinical abnormality presented by the rats. Moreover, the biochemical, haematological, and urine analysis data were in the same trend as the vehicle-treated rats, and histopathological alterations in the selected organs were not observed. The study revealed that the lemongrass leaves extract obtained by the UAE technique is safe with LD_{50} > 5000mg/kg bw.t.

Keywords: Sprague Dawley, lemongrass, Ultrasound-assisted extraction, Cymbopogon citratus, toxicity.

INTRODUCTION

In Asia, Africa, and South America, lemongrass, botanically called Cymbopogon citratus, is a perennial herbaceous medicinal plant with slender, thin leaves that is extensively grown for its essential oils. This aromatic plant is a member of the Poaceae family. The essential oils obtained from this plant are reported to have antibacterial, antifungal, and anticonvulsant activities (Ashaq *et al*., 2024). Several other studies have reported that lemongrass essential oils have anticarcinogenic, anti-inflammatory, antidiabetic, antidyslipidaemic, and antiprotozoal properties and can inhibit platelet aggregation (Falah *et al*., 2015; Lulekal *et al*., 2019a; Sunarwidhi *et al*., 2022). Ashaq *et al*. (2024) stated that lemongrass essential oils have an oral antifungal activity against candida in HIV-infected patients. In the manufacturing of soaps, detergents, perfumes, and body creams, lemongrass essential oils are used as fragrances. Aside from that, it is also used as a flavouring and preservative agent in the manufacturing of non-alcoholic beverages and baked foods (Lulekal *et al*., 2019a).

The extractive method influences the type and rate of obtaining bioactive compounds from natural products. Routinely, the extraction of phytochemicals from plant materials is done utilising the solvent extraction method (commonly used solvents: ethyl acetate, methanol, acetone, and ethanol), hydro distillation (HD), steam distillation (SD), empyreumatic distillation, and maceration (Ranitha *et al*., 2014). The techniques stated above are reported to be ineffective and induce hydrolysis, thermal degradation, and solubilization of some bioactive metabolites extracted (Irfan *et al*., 2022; Suryawanshi *et al*., 2016). It has been reported in previous studies that the quality of the phytochemical constituents extracted is primarily influenced by the extraction method used. A report by Mukarram *et al*. (2021) indicates that monoterpenes in lemongrass undergo considerable chemical changes by steam distillation. Also, essential oils extracted by solvent extraction methods contain residues that pollute the fragrances and lead to the loss of volatile compounds when the solvent is evaporated (Ranitha *et al*., 2014a, 2014b). However, modern extraction technologies such as pulsed electric field, microwave-assisted, enzyme-assisted, subcritical fluid, supercritical fluid, high pressure, and ultrasonication assistive methods have been developed to improve upon the efficiency of extraction, operational cost, shorten the extraction time, and improve extraction yield (Ranjha *et al*., 2021; Suryawanshi *et al*., 2016). Ultrasound-assisted extraction (UAE) is a promising technique of extraction because it is relatively safe, time-saving, utilises a small amount of energy, and ensures exhaustive extraction of bioactive principles (Anh *et al*., 2021). Moreover, the application of lower temperatures in the UAE prevents thermal degradation of bioactive compounds. Ultrasound-induced acoustic cavitation disrupts the cell walls by mass heat and solvent penetration (Shen *et al*., 2023; Yaa *et al*., 2025). Optimising the ultrasound operating parameters (such as polarity, pH, time, solvent, etc) improves the extraction efficiency and quality of yield. Several studies have reported the advantages of the ultrasonic extraction technique over other conventional methods, however, there are no reports of in vivo toxicity studies of UAE ethanolic lemongrass leaves extract in SD rats. Earlier studies focused on the comparison between the yield and antioxidant activities of the phytochemicals extracted by ultrasonication and conventional extraction methods. To fully understand the efficiency of the ultrasonication method, it is important to carry out in vivo acute and sub-chronic toxicity studies of UAE ethanolic lemongrass extract to

evaluate the clinical safety of extracts obtained by this method. Thus, this study was designed to evaluate the acute and sub-chronic toxicity of UAE ethanolic lemongrass leaves extract in SD rats.

MATERIALS AND METHODS

Plant materials

Fresh leaves of lemongrass were sampled at Taifa-Burkina in the Greater Accra Region in October 2022. The plant was authenticated by a plant scientist and a voucher specimen (CPMR5160) was stored at the herbarium of the Centre for Plant Medicine Research (CPMR).

Sample preparation

The harvested lemongrass was washed, air-dried, and pulverised into powder, bagged in sealed airtight plastic bags, and stored in a dry cabinet. The ethanolic crude extract of the lemongrass was obtained by ultrasonication as described below.

Ultrasound-Assisted Extraction (UAE) of ethanolic lemongrass extract

The extraction of UAE ethanolic lemongrass extract was done as described by (Bimakr *et al*., 2013; Sheng *et al*., 2014) with slight modifications due to differences in solvent composition, spec of the instrument, and the sample material. A 25g of pulverised lemongrass powder was weighed with an electronic balance (Golden-Mettler, USA) into a 500 ml Erlenmeyer flask, and 250 ml of 70% ethanol was added. The aluminium foil was used to wrap the flask to prevent ethanol from evaporating. The extraction was performed using an ultrasound cleaner (Cole Parmer Ultrasonic Cleaner, Model 08895-04, American Instrument Exchange Inc., USA). The flask was immersed in an ultrasonic cleaner filled with water to the maximum level. The plant material was subjected to ultrasonic waves at 70°C for 40min with a frequency output of 40 kHz. The mixture obtained was sieved with a white cloth and then with a Whatman No. 1 filter paper with pores 110 diameter. The ethanol in the crude filtrate was evaporated using RE-52A, E. Track Scientific Instruments, England rotary evaporator at 78°C and dried at 70°C in a water bath. The dried extracts were stored in a freezer at -20°C until use.

Ethical considerations

The Research and Ethical Review Committee of the Faculty of Applied Sciences of the Accra Technical University approved the study to be conducted after a scientific and institutional review board (IRB) reviewed and approved the protocol (ATU/MLT/ET/011903 01B/908745D/2021-2022). The SD rats were provided with water and food ad libitum, and the bedding of the animals was cleaned out every other day to minimise suffering. They were allowed to sleep in a dark and calm environment and monitored for any sign of distress or discomfort over the study period. The ARRIVE 2.0 reporting standards for reporting animal research (du Sert *et al*., 2020) and internationally accepted principles and guides for caring and use of laboratory animals, according to the United States National Research Council (USNRC) were followed (NRC, 2011).

In vivo studies

Sample size calculations

Based on the resource equation approach, the sample size computation was done as follows (Arifin & Zahiruddin, 2017; Charan & Kantharia, 2013).

$$n = \frac{D}{e} + 1 \qquad \textbf{eqn 1}$$

where n = number of rats per group, D = degrees of freedom (10 – 20), e = number of groups. After the computation of the sample size, a sample size of range between 4 – 6 rats per each of the four groups was arrived at. Thus, to increase the accuracy and power of the study, 10 rats per group and 6 rats per group were used for the acute and subchronic studies, respectively.

Experimental Animals

Healthy 60-day-old female SD rats (nulliparous and non-pregnant) weighing between 157-183g were bought from the Centre for Plant Medicine Research (CPMR) for this study. According to Test No. 423 and 408 of the OECD guidelines, female rats were selected for the study because of their higher sensitivity to toxicants than male rats (OECD, 2001a, 2018). The rats were kept with a 12-hour light/dark cycle and humidity at a room temperature of 23 to 25ºC (Donkor *et al*., 2014). The rats were fed with rats' chow (17.09% crude protein, 3.368% ether extract, 3.35% crude fibre, 3.0% calcium, 0.56% phosphorus, 0.76% lysine, 0.46% methionine, 2,873.4 kcal/kg of metabolised energy) purchased from Agro Food Company and unlimited access to water. The rats were allowed to acclimatise in the groups for one week before the start of the study.

Acute Toxicity Study

Acute toxicity assessment of the UAE ethanolic lemongrass leaves extract was conducted using the fixed-dose procedure by the Organisation for Economic Cooperation and Development (OECD) test no. 420 guidelines (OECD, 2001b). Non-pregnant and nulliparous SD rats (Healthy females) were randomized into two groups (n = 10) and allowed 1-week period of acclimatisation. The SD rats were ad libitum allowed access to water and fasted overnight. The median lethal (LD_{50}) dose of the UAE ethanolic lemongrass leaves extract was determined by orally administering a 5000mg/kg bw.t dose of the extract to the rats in the test group and the control group with 2ml/kg bw.t normal saline as a control using an oesophageal cannula. The rats were monitored every hour for the first 12 hours for mortality and any abnormal physiological or behavioural changes for the next 24 hours, through fourteen days. The evaluation of signs of toxicity was conducted using the Irwin test, the primary observation protocol (Irwin, 1968).

Sub-chronic Toxicity Study

Sixty (60) days-old female (non-pregnant and nulliparous) SD rats were randomised into groups of six (n = 6) and permitted 1 week of acclimatisation before the study. Water and rats' chow were provided to the rats ad libitum. The groupings for the study were:

Group 1- water (control)

Group 2- 200mg/kg bw.t (Low dose)

Group 3- 600mg/kg bw.t (Medium dose)

Group 4- 1200mg/kg bw.t (High dose)

The exact doses of the extracts were administered daily to the rats for a six-week duration. The control group was provided with sterilised distilled water. Following the Irwin test procedure, any abnormal physical appearance, water intake, food intake, and behavioural changes were recorded (Irwin, 1968). The rats' body weights were recorded at baseline, 2nd, 3rd, 4th, 5th, and 6th weeks. Urine and blood samples were sampled at baseline, 3rd, and 6th weeks for analysis, and histopathological analysis of the kidney, liver, heart, lungs, and spleen was conducted at the termination of the study. The OECD Test No. 408 (OECD, 2018) was followed for the conduct of the sub-chronic toxicity assessment.

Urinalysis

The rats' bladders were manually expressed by the application of gentle trans-abdominal pressure, and clean and fresh urine samples were delivered onto a tabletop (Kurien *et al*., 2004). To avoid cross-contamination, the immersed urine dipsticks were blotted and dried with soft tissues. A urine colour chart was compared with the urine strips for colour development. The urine of the rats was collected and analysed at baseline, 3rd, and 6th weeks for proteins, glucose, urobilinogen, nitrite, leucocytes, bilirubin, pH, specific gravity, blood, and ketones with Urit urine reagent strips (Urit Medical Electronics Co. Ltd, China).

Blood Sample collection and excision of essential organs

By the tail straining method, the rats' blood samples were harvested at baseline, 3rd, and 6th weeks into gel separator tubes (GST) for biochemical analysis and EDTA tubes for haematological analysis. The blood samples in the GST were permitted to clot for 20 minutes before spinning at 5000g for 5 minutes with a centrifuge, and the serum was harvested into labelled Eppendorf tubes. The serum was stored -20°C until analysis. The EDTA tube samples were gently inverted about 10 times and twirled to enhance the homogenous mixing of the EDTA with the blood to prevent clots.

The experiment was terminated on the 42nd day of the study, and by cervical dislocation, after the rats had been chloroformed, they were sacrificed and dissected. Essential organs excised include the liver, heart, lungs, kidneys, and spleen. They were washed with normal saline to remove fat and connective tissues. The washed organs were bloated and dry, and their weights were recorded with an electronic balance. Ten percent neutral buffered formalin (pH=7.2) was used to fix the tissues (Abotsi *et al*., 2011).

Haematological Analysis

The full blood count parameters such as White Blood Cells (WBC), Lymphocyte (Lymph#), Granulocyte (Gran#), Red Blood Cells (RBC), Haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH), Haematocrit (HCT), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Red Cell Distribution Width (RDW), Platelet Distribution Wide (PDW), Platelet (PLT), Mean Platelet Volume (MPV), Platelet crit (PCT) were measured with BC-10 fully automated Mindray Haematology analyzer (Shenzhen Mindray Bio-Medical Electronics Co. Ltd, China).

Serum Biochemical Analysis

A serum biochemical examination of the adverse effects of the extract on the renal and hepatic function was conducted. albumin (ALB), Total protein (TP), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), total and direct bilirubin (BIT, BID), were the measured biomarkers of liver function. Also, serum creatinine (Crea), urea, sodium (Na^+), chloride (Cl^-), and potassium (K^+) were also measured as biomarkers of renal function with BS-240 fully automated Shenzhen Mindray chemistry analysers.

Percentage of body weight gain and relative organ weight

The rats' body weights were recorded at baseline, 3rd, and 6th weeks of the experiment and the organ weights were recorded at termination of the study. The percentage gain in body weight (%) during the study period was computed as follows using equation (2)

$$\text{Weight gain (\%)} = \frac{W(g) - Wb(g)}{Wb(g)} x\,100\,\% \,. \qquad \textbf{eqn 2}$$

Where W= Mean weekly rat's body weight, Wb = mean baseline rat body weight.

The relative organ weights were computed as shown in equation (3)

$$\text{ROW} = \frac{Wo(g)}{Wf(g)} x\,100\,\% \,. \qquad \textbf{eqn 3}$$

Where ROW= relative organ weight, Wo= organ weight of rats, and Wf = final body weight of rats at termination of the experiment.

Histopathological examination

Neutral buffered formalin (10%) fixed liver, kidney, spleen, lungs, and heart tissues were histologically examined to determine the effect of the UAE ethanolic lemongrass extract on the cells and tissues of these essential organs. The tissues were dehydrated through ascending grades of ethanol solutions (70%, 80%, 90%, and absolute). After paraffin wax embedding, they were processed for histopathological analysis. Cut sections of these tissues (2µm thickness) were stained with haematoxylin and eosin (H&E) and mounted for examination with an Olympus microscope and images were taken (Abotsi *et al*., 2011).

Statistical analysis

Microsoft Excel 365 was used for data cleaning and statistical analysis. The findings of the study were summarised as mean ± standard error of the mean (SEM) and presented in tables or plotted as charts. One-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test, was performed with GraphPad Prism version 8.4.2 (GraphPad Software, San Diego, CA, USA), and $P < 0.05$ was considered statistically significant.

RESULTS

Acute toxicity study

Acute toxicity of the UAE ethanolic lemongrass leaves extract in SD rats was determined using the single-dose procedure. Ten (10) SD rats, after the oral administration with a single dose of 5000mg/kg bw.t UAE ethanolic lemongrass leaves extract, were monitored for signs of toxicity immediately and up to 12 hours, 24hours, 48hours, 7 days, through to 14 days after the treatment. Signs of acute toxicity such as salivation, diarrhoea, locomotory defects, lachrymatory, increased urination, piloerection, difficulty in breathing, and asthenia were not recorded over the study period. No death was also recorded. Hence, the LD_{50} of UAE ethanolic lemongrass leaves extract was greater than 5000mg/kg bw.t.

Sub-chronic toxicity study

Percentage of body weight gain and relative organ weight

The effect of UAE ethanolic lemongrass leaves extract on the percentage body weight change (%) of Sprague Dawley rats on daily administration of the extract for six weeks is shown (Figure 1). Both control and test animals steadily gained weight from the 2nd to the 6th week. The weight gained by the control and the 200mg/kg bw.t treatment rats was relatively the same, which was higher than the 600mg/kg bw.t and 1200mg/kg bw.t treatment groups. The weight gained in the control, 200mg/kg bw.t, and 600mg/kg bw.t was steady; however, there was fluctuation in the weight gained in 1200mg/kg bw.t treatment from week 4 to week 6, with week 4 being the highest and week 5 being the least. The area under the curve (AUC) analysis also reveals the same trend that the 200mg/kg treatment group gained more weight than the control, followed by the 1200mg/kg and 600mg/kg being the least; however, this percentage change in body weight was not statistically significant ($p > 0.05$) (Figure 2).

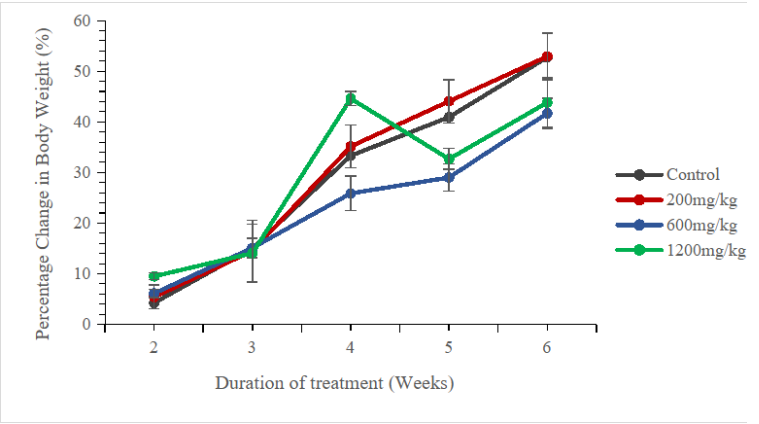

Figure 1: A plot of the percentage body weight change of SD rats over the six-week study period. The data points represent the mean ± SEM (n = 6).

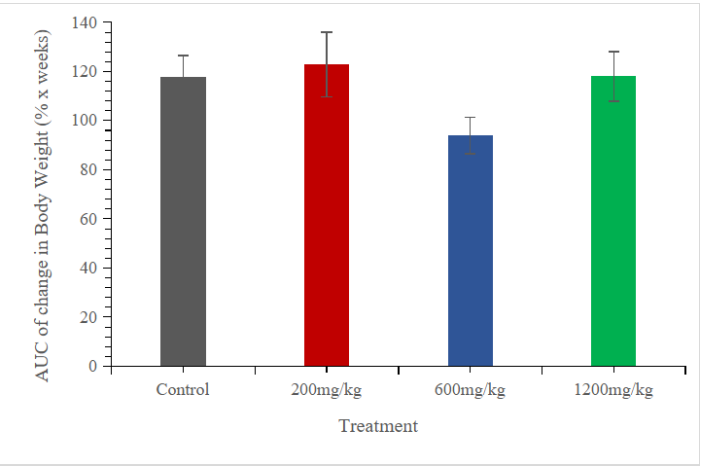

Figure 2: Mean area under the curve (AUC ± SEM) of the percentage body weight change line graph for each group represented by bar chart.

Effect of UAE ethanolic lemongrass leaves extract on percentage organ/ weight

Table 1 shows the percentage of organ/ body weight of the rats after 6 weeks of oral treatment with UAE ethanolic lemongrass leaves extract. The results show that the percentage of organ/body weight was not significantly different ($p > 0.05$) between the test groups and control groups. Also, it was comparatively the same between the control and the treatment groups and among the treatment groups.

Table 1. Effect of UAE ethanolic lemongrass leaves extract on the percentage organ/body weight at the 6th week of the experiment in SD rats

Experimental Group (mean ± SEM, (n = 6)) UAE Ethanolic Lemongrass extract					
Organ	**Control**	**200mg/kg**	**600mg/kg**	**1200mg/kg**	**P-value**
Kidney	0.52 ± 0.02	0.59 ± 0.04	0.52 ± 0.03	0.58 ± 0.01	>0.05
Liver	2.36 ± 0.06	2.57 ± 0.12	2.66 ± 0.15	2.64 ± 0.17	
Spleen	0.26 ± 0.04	0.27 ± 0.01	0.22 ±0.01	0.24 ± 0.01	
Lungs	0.52 ± 0.02	0.67 ± 0.03	0.63 ± 0.08	0.73 ± 0.10	
Heart	0.40 ± 0.12	0.35 ± 0.03	0.28 ± 0.01	0.32 ± 0.02	

Effect of UAE ethanolic lemongrass leaves extract on haematological parameters in SD rats in the 6th week of the experiment

Table 2 below shows the effect of UAE lemongrass leaves extract on haematological parameters in Sprague Dawley rats. WBC ($18.6x10^9$)/L, Lymph# ($11.98x10^9$)/L, Mid# ($1.50x10^9$)/L, and Gran% (23.58%) were elevated in the control animals than the test animals WBC (10.36 – 12.90)109/L, Lymph# (7.38 – 9.73)10^9/L, Mid# (0.85 – 1.08)10^9/L, Gran# (2.10 – 2.40) and Gran% (16.95 – 22.35%). The ANOVA results showed no significant differences between the test animals and the control groups.

Table 2: Effect of UAE ethanolic lemongrass leaves extract on the 6th week haematological parameters in SD rats

Experimental Group (mean ± SEM of n = 6) UAE lemongrass leaves extract					
Parameter	**Control**	**200mg/kg**	**600mg/kg**	**1200mg/kg**	**P-value**
WBC ($x10^9$/L)	18.6 ± 5.21	12.90 ± 1.80	11.50 ± 0.89	10.63 ± 0.96	>0.05
Lymph# ($x10^9$/L)	11.98 ± 2.43	9.73 ± 1.49	8.05 ± 0.52	7.38 ± 0.79	
Mid# ($x10^9$/L)	1.50 ± 0.35	1.08 ± 0.18	1.08 ± 0.21	0.85 ± 0.13	
Gran# ($x10^9$/L)	5.13 ± 2.55	2.10 ± 0.19	2.38 ± 0.25	2.40 ± 0.63	
Lymph (%)	68.05 ± 4.58	74.80 ± 1.64	69.95 ± 1.69	69.60 ± 5.223	
Mid (%)	8.38 ± 0.51	8.25 ± 0.21	9.10 ± 0.98	8.05 ± 0.83	
Gran (%)	23.58 ± 4.89	16.95 ± 1.62	20.95 ± 1.42	22.35 ± 4.46	
RBC ($x10^{12}$/L)	8.11 ± 0.27	8.95 ± 0.30	8.60 ± 0.20	8.47 ± 0.34	
HB (g/dL)	16.70 ± 0.60	17.18 ± 0.30	16.35 ± 0.18	16.23 ± 0.17	
HCT (%)	50.48 ± 1.15	50.75 ± 1.41	49.93 ± 0.68	48.38 ± 0.45	
MCV (fL)	56.83 ± 1.76	56.78 ± 1.52	58.13 ± 0.68	57.35 ± 1.68	

MCH (pg)	18.78 ± 0.71	19.25 ± 0.46	19.05 ± 0.35	19.30 ± 0.63
MCHC (g/dL)	33.03 ± 0.43	33.93 ± 0.50	32.75 ± 0.25	33.58 ± 0.21
RDW-CV (%)	17.63 ± 0.42	17.80 ± 0.60	18.50 ± 0.65	17.98 ± 0.27
RDW-SD (fL)	33.33 ± 1.41	33.60 ± 1.43	35.70 ± 1.23	34.23 ± 0.91
PLT (x10^9/L)	772.30 ± 60.25	733.50 ± 28.86	823.25 ± 38.62	700.25 ± 70.43
MPV (fL)	6.63 ± 0.23	6.55 ± 0.10	6.83 ± 0.11	6.60 ± 0.19
PDW	14.58 ± 0.09	14.50 ± 0.08	14.58 ± 0.03	14.43 ± 0.10
PCT (%)	0.51 ± 0.04	0.48 ± 0.02	0.56 ± 0.03	0.46 ± 0.05
P-LCR	0.08 ± 0.01	0.07 ± 0.01	0.082 ± 0.01	0.07 ± 0.01

Effect of UAE ethanolic lemongrass leaves extract on liver function test in SD rats

Figure 3 shows the effect of six weeks of gavage of UAE lemongrass leaves extract on liver function tests in SD rats. The ALT activities in the respective treatment groups were comparable to those of the control group and among the treatment groups. Similarly, the observation with GGT activity was not different. However, the ALP activity for the 200mg/kg bw.t test group was elevated than both the 600 and 1200mg/kg bw.t and the control groups. The control group activity was also marginally higher than the 600 and 1200mg/kg bw.t treatment groups, and the 600mg/kg bw.t treatment group was also higher than the 1200mg/kg bw.t treatment group. Similarly, the AST activity, the 1200mg/kg bw.t activity, was marginally higher than the control, and both were marginally higher than the 200 and the 600mg/kg bw.t groups. No statistically significant difference between the control and treatment groups and among the treatment groups was detected ($p > 0.05$).

The total bilirubin concentration was higher than the indirect, which was also higher than the direct bilirubin concentration. The total bilirubin concentration for the 1200mg/kg bw.t group was higher than the control, 200, and 600mg/kg bw.t groups. However, the bilirubin concentration of the control, 200, and 600mg/kg bw.t treatment groups was comparatively the same. The differences between the test and the control groups and among the test groups were not statistically significant. A concentration-dependent decrease in direct bilirubin concentration was observed; however, this was not statistically significant. The indirect bilirubin concentration was higher in the control group, followed by the 1200mg/kg bw.t. and the 200 and 600mg/kg bw.t indirect bilirubin concentrations were comparatively the same and lesser than the control and 1200mg/kg bw.t groups. It was observed that the total protein and albumin concentrations were comparatively the same between the control and the test groups and among the test groups.

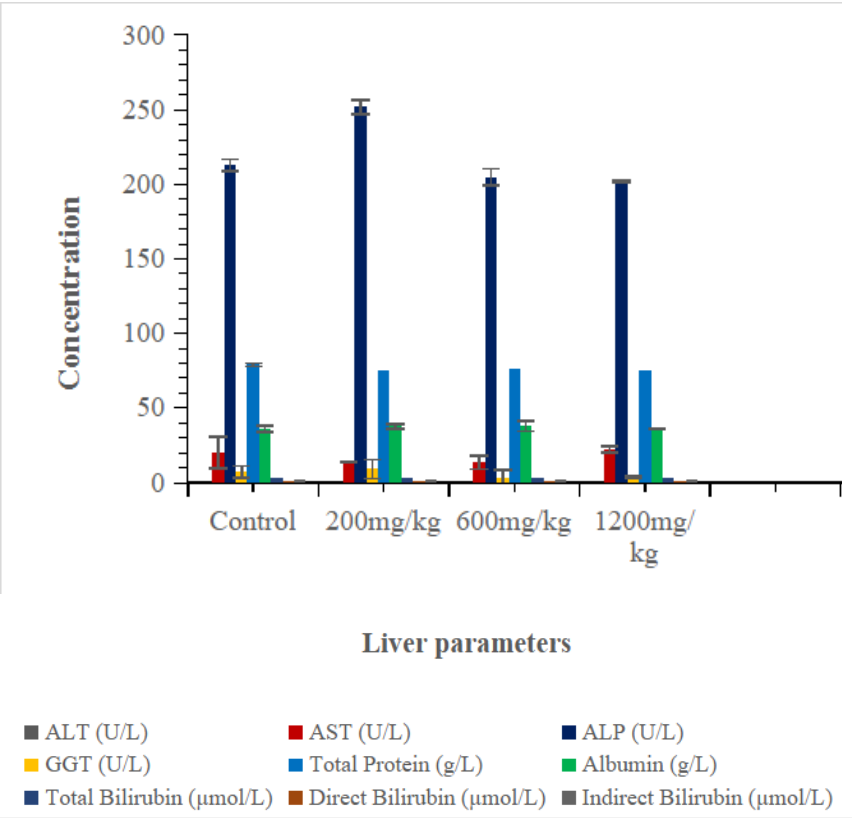

Figure 3: Effect of UAE ethanolic lemongrass leaves extract on serum liver function test at the 6th week of the experiment in SD rats. Each bar represents mean ± SEM (n = 6), ($p > 0.05$). ALP- alkaline phosphatase, ALT- alanine aminotransferase, AST- aspartate aminotransferase, and γ-GT- gamma-glutamyl transferase.

Effect of UAE ethanolic lemongrass leaves extract on renal function in SD rats

Figure 4 represents the effect of UAE ethanolic lemongrass leaves extract on renal function tests in SD rats after six weeks of daily oral administration of the extract. It was observed that the serum concentration of urea was comparatively the same between the control and the treatment groups and among the treatment groups. There was a concentration-dependent decrease in the creatinine concentration (control > 200mg/kg > 600mg/kg > 1200mg/kg). This was, however, not statistically significant. The concentrations of sodium between the test and control groups and among the test groups were almost the same. Similar trends were observed for chloride and potassium concentrations. No statistically significant differences were detected ($p > 0.05$).

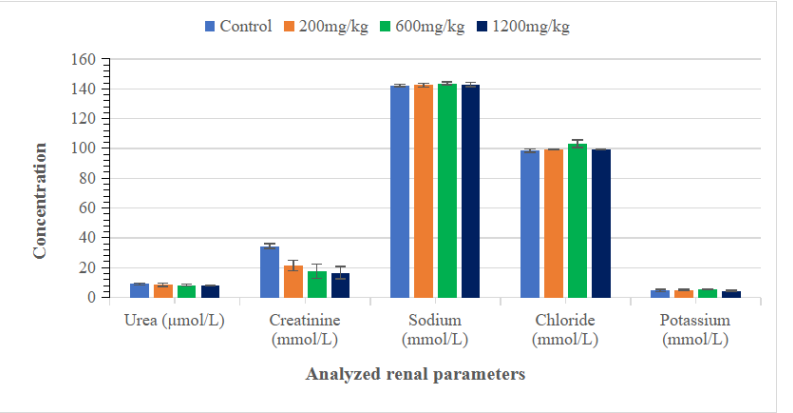

Figure 4: Effect of UAE ethanolic lemongrass leaves extract oral daily administration on sodium, chloride, and potassium concentration at the 6th week of the experiment in SD rats. Each bar is the mean ± SEM (n = 6), ($p > 0.05$).

Effect of UAE ethanolic lemongrass leaves extract on SD rats' urine parameters at the 6th week of the experiment

Table 3 shows the results on the effect of UAE ethanolic lemongrass leaves extracts on urine parameters of SD rats. No abnormality was detected.

Table 3: Effect of UAE ethanolic lemongrass leaves extract on urine parameters in the 6th week of the experiment in SD rats

Experimental Group UAE ethanolic lemongrass leaves extract

Parameters	Control	200mg/kg	600mg/kg	1200mg/kg
Bilirubin (mg/dL)	--	--	--	--
Glucose (mg/dL)	--	--	--	--
Ketones (mg/dL)	--	--	--	--
Blood	--	--	--	--
Specific Gravity (g/mL)	1.020 ± 0.005	1.015 ± 0.000	1.020 ± 0.000	1.016 ± 0.005
pH	7.38 ± 0.25	7.50 ±0.41	7.00 ± 0.00	7.50 ±0.41
Protein (g/L)	--	--	--	--
Leukocyte (µL)	--	--	--	--
Nitrite	--	--	--	--
Urobilinogen (mg/dL)	n	n	n	n

Each data point is the means ± SEM of n = 6, (--): Absent, (n): Normal

Gross pathology and histopathological examination

Figures 5-9 shows the histopathological results of the kidney, heart, liver, lungs, and spleen tissues after 6 weeks of gavage of UAE ethanolic lemongrass leaves extract. The gross pathology and histopathological assessment of the heart, spleen, kidney, liver, and lung tissues revealed that they were normal in all groups. The results show that there was no alteration in the tissue's appearance and architecture. No histopathological lesion, cellular swelling, cytoplasmic fragmentation, myocardial infarct and inflammatory cells, tubular atrophy, steatosis, or abnormal cellularity were observed.

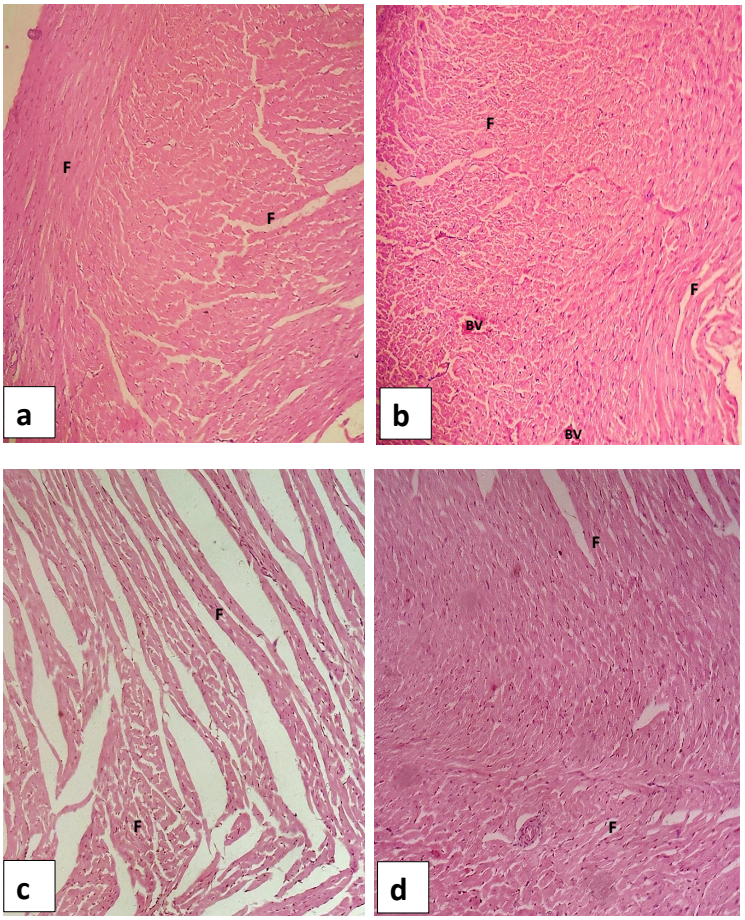

Figure 5. Photomicrographs showing histopathological examination of haematoxylin eosin-stained representative heart tissues after six weeks of oral gavage of UAE ethanolic lemongrass leaves extract in Sprague Dawley rats. Micrographs show the distinct myocardial fibres (F) arrangement in longitudinal and transverse sections with centrally placed nuclei and blood vessels (BV).

No myocardial infarcts and inflammatory cells are present. (a) control group administered with sterilised distilled water (Mag: x300). (b) 200mg/kg bw.t (Mag: x300). (c) 600mg/kg bw.t (Mag: x300). (d) 1200mg/kg bw.t (Mag: x300).

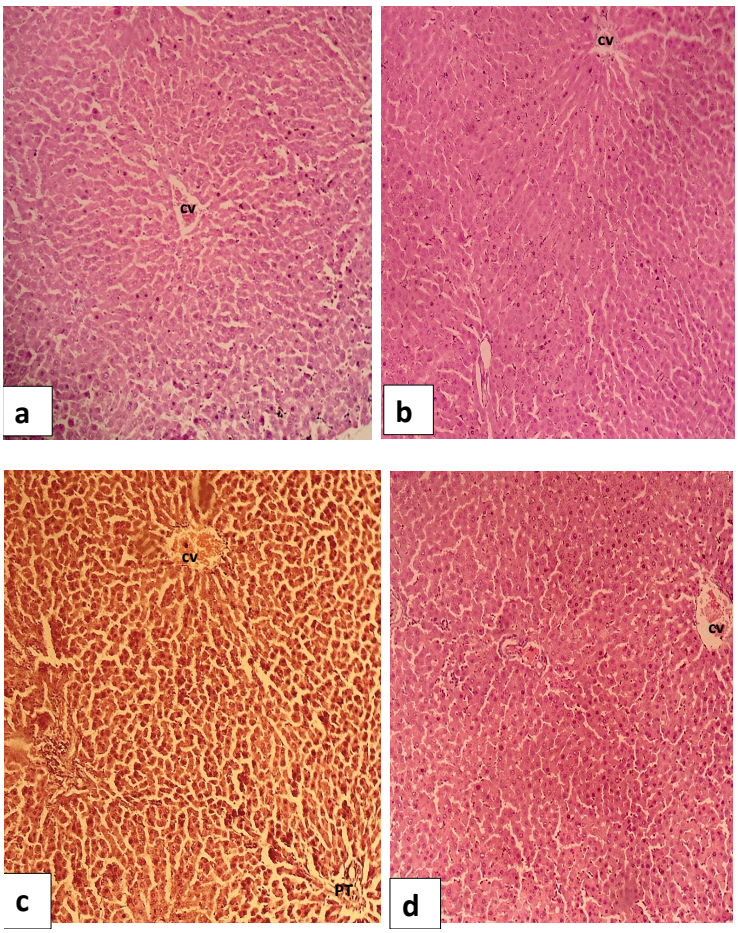

Figure 6. Photomicrographs showing histopathological examination of haematoxylin eosin-stained representative liver tissues after six weeks gavage of UAE ethanolic lemongrass leaves extract in SD rats. The photomicrographs revealed the central veins (CV) surrounded by radial plates of hepatocytes. Blood vessels such as the portal triads (PT) with a branch of the portal vein, bile duct, and hepatic artery. Neither necrosis nor inflammatory cells were present in treatment groups including the control group. (a) control group administered with sterilised distilled water (Mag: x300). (b) 200mg/kg bw.t (Mag: x300). (c) 600mg/kg bw.t (Mag: x300). (d) 1200mg/kg bw.t (Mag: x300).

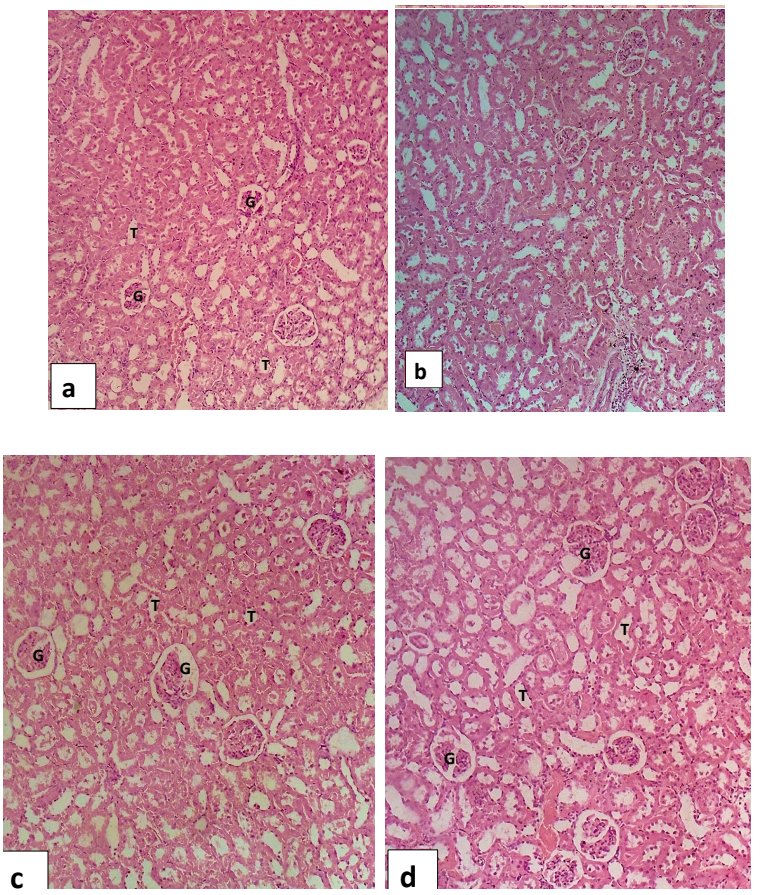

Figure 7. Photomicrographs showing histopathological examination of haematoxylin eosin-stained representative kidney tissues after six weeks of gavage of UAE ethanolic lemongrass leaves extract in SD rats. Renal tubules (T) and glomeruli (G) epithelial cells made up of single layers and regular nuclei were observed in the photomicrographs. No interstitial fibrosis, inflammatory infiltrates or tubulartrophy were observed. (a) control group administered with sterilised distilled water (Mag: x300). (b) 200mg/kg bw.t (Mag: x300). (c) 600mg/kg bw.t (Mag: x300). (d) 1200mg/kg bw.t (Mag: x300).

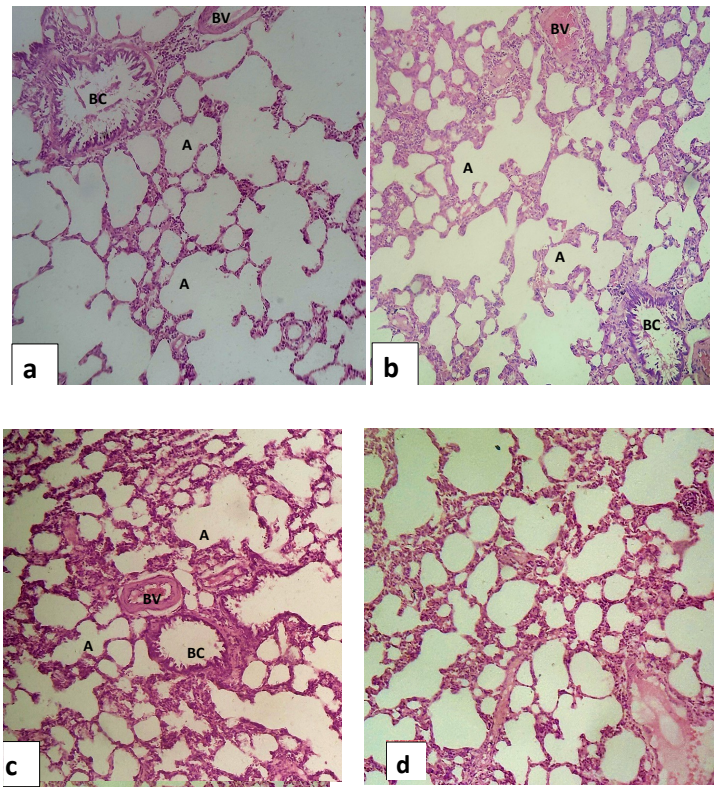

Figure 8. Photomicrographs showing histopathological examination of haematoxylin eosin-stained representative lung tissues after six weeks gavage of UAE ethanolic lemongrass leaves extract in SD rats. It was observed from micrographs that lung architecture was normal with bronchiole (BC), blood vessels (BV), and alveolar spaces (A) with no significant neoplastic changes or inflammation in all groups. (a) control group administered with sterilised distilled water (Mag: x300). (b) 200mg/kg bw.t (Mag: x300). (c) 600mg/kg bw.t (Mag: x300). (d) 1200mg/kg bw.t (Mag: x300).

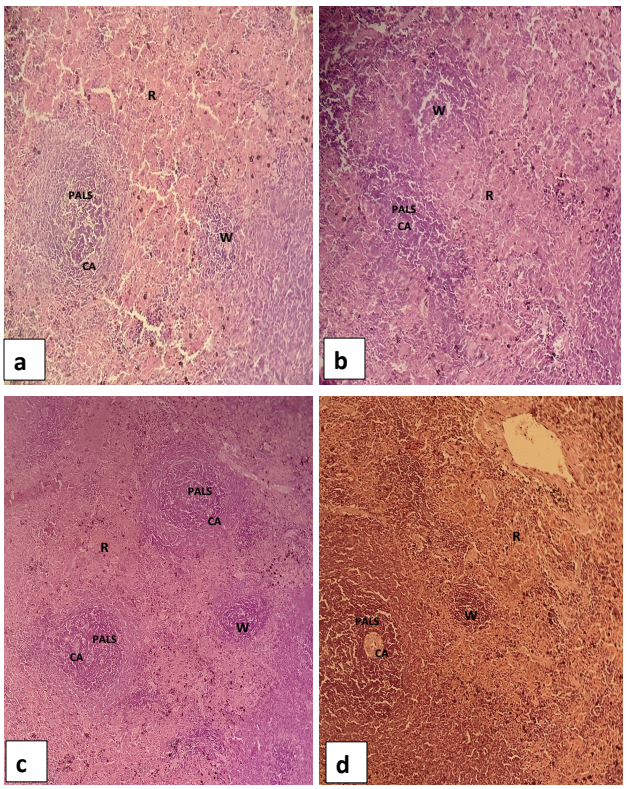

Figure 9. Photomicrographs showing histopathological examination of haematoxylin eosin-stained representative spleen tissues after six weeks of gavage of UAE ethanolic lemongrass leaves extract in SD rats. The photomicrographs show the white pulp (W) consisting of lymphoid tissue with periarteriolar lymphoid sheaths (PALS) flanking the central arterioles (CA). The reddish pulp (R) regions are splenic cords consisting of macrophages, reticular fibres, red blood cells, and venous sinuses. Neither fibrosis, abnormal cellularity, nor inflammation was observed. (a)The control group was administered with sterilised distilled water (Mag: x300). (b) 200mg/kg bw.t (Mag: x300). (c) 600mg/kg bw.t (Mag: x300). (d) 1200mg/kg bw.t (Mag: x300).

DISCUSSION

Safety assessment of any herbal plant is purposefully carried out to identify adverse side effects of the plant and to determine the dose at which the adverse side effects would be observable (Ibrahim *et al*., 2016; Ugwah-Oguejiofor *et al*., 2019). It was observed from the acute toxicity studies that the UAE ethanolic lemongrass leaves extract orally gavaged to the SD rats did not stimulate any sign of behavioural, motor, or neuronal changes. No morbidity or mortality was recorded in any of the groups within the study period (14 days). This indicates that the LD50 of UAE ethanolic lemongrass leaves extract may be greater than 5000mg/kg bw.t. According to the OECD's Globally Harmonized Classification System (GHS), mixtures and chemical substances with LD_{50} > 200-5000mg/kg are classified as category

5 substances (the lowest toxicity category) for acute oral toxicity (OECD, 2001c). This signifies that if ingested orally at a dose below the limit indicated in the classification range, it is not anticipated that such substances will be acutely hazardous. This suggests that UAE ethanolic lemongrass leaves extract is safe when acutely consumed at concentrations not >5000mg/kg bw.t. Our result is in comparison with other reported literature which says the LD_{50} of lemongrass extract is >5000mg/kg bw.t (Lulekal *et al*., 2019b; Tarkang *et al*., 2012; Tarkang *et al*., 2012; Xavier *et al*., 2022). It thus appears that different solvents and extraction methods do not alter or affect the level of acute toxicity of lemongrass extracts. Acute toxicity studies were performed to determine the dose range that subsequent testing can use. It is also an important marker for the determination of the therapeutic index of drugs and xenobiotics (i.e. LD_{50}/ED_{50}) (Aniagu *et al*., 2005). However, bioaccumulation of low-dose toxic substances could later cause clinical hazards to the exposed subjects that could not have been detected acutely. Due to the limited clinical application of acute toxicity studies, sub-chronic toxicity experimentation was relevant for a detailed safety evaluation of the extract. The sub-chronic toxicity evaluation of the extract was conducted after oral gavage of 200, 600, and 1200 mg/kg bw.t of the UAE ethanolic lemongrass leaf extract, respectively, for six weeks and the effect on haematological, body weight, biochemical, and histopathological indices was recorded. Decline of an individual's body weight is an important clinical indicator of deterioration of one's health, even before major systemic damage is apparent. It was observed that the daily gavage of the extract to the rats produced no significant weight loss in the treatment groups. The analysis of the area under the curve revealed that, except for the 600mg/kg bw.t group, whose weight was not significantly less than the control group, the 200 and 1200mg/kg bw.t groups' body weight gained was not different from the control (Figure 2). The selective reduction in weight in the 600mg/kg would likely be due to changes in food intake rather than metabolic effects. Implying that the extract might be inducing appetite loss at this concentration of the extract rather than directly altering their metabolism (Rahman *et al*., 2017). The findings suggest that the UAE ethanolic lemongrass leaves extract had no harmful effect on the growth and health of the test animals.

The Society of Toxicological Pathology (STP) recommends the organ/body weight ratio as a critical tool for the assessment of organ-specific toxicity. Even though the organ weight alone could be used, the calculation of the organ/body weight ratio helps normalise the variability due to body weight and feeding fluctuations in studies with anti-obesity test articles (Sellers *et al*., 2007). The liver weight to body weight ratio is a significant indicator of hepatocellular hypertrophy, lipidosis, or peroxisome proliferation, and metabolic and physiologic perturbations (Michael *et al*., 2007). An increase in the kidney weight to body weight ratio is a sensitive marker of acute renal injury, tubular hypertrophy, or progressive nephropathy. Also, elevated heart weight may suggest myocardial hypertrophy, which in most cases is microscopically and macroscopically difficult to identify (Michael *et al*., 2007; Sellers *et al*., 2007). These reasons thus justify the organ-to-body weight ratio as an important parameter for investigating the adverse side effects of pharmaceutical, medical, and chemical test articles. The study revealed that the organ-to-body weight ratio was comparatively similar for the test and control animals (Table 1). This suggests that there are no severe adverse effects of the extract on the studied organs. These results of the organ/body weight ratio corroborate the histopathological data, which show that there was no morphological and histological alteration of the tissues of the studied organs.

This indicates that the UAE lemongrass leaves extract may have no organ-specific toxic effect in the SD rats at doses, 1200mg/kg bw.t or below. The findings from this study corroborate Ayenew *et al*. (2022), according to whom the organ-to-body weight ratio of mice treated with C. martini essential oils had no appreciable variations between the test and the control animals.

Due to the interaction of the circulatory system with drugs and xenobiotics, blood is a vital tool used in assessing the physiological and pathological state of vertebrates. Haematological parameters change relative to the state of health of the organism, which may be due to exposure to toxicants, metabolism, cellular integrity, and membrane permeability. In this study, no significant effect of the UAE ethanolic lemongrass leaves extract on haematological parameters was recorded (Table 2). The study revealed that the treatment groups had a non-significant decrease in WBC count, lymphocytes, and granulocyte numbers relative to the control (Table 2). This suggests that UAE ethanolic lemongrass extract may have anti-leucocytic, anti-lymphocytic, and anti-granulocytic activity. The haemoglobin and RBC differentials were similar for both control and tested groups, suggesting that the extract does not affect iron synthesis and thus haemoglobin production in the blood. It is also possible that lemongrass does not quantitatively contain high concentrations of saponins, which are known to have deleterious haemolyzing effects on circulating erythrocytes(Omotayo *et al*., 2024). On the other hand, the platelet count of the control group was elevated than the 200mg/kg bw.t and 1200mg/kg bw.t but lower than the 600mg/kg bw.t treatment group. Thus, the UAE ethanolic lemongrass leaves extract may not contain bioactive principles with thrombocytosis activity. In developing countries, liver disorders major cause of morbidity and mortality (Devarbhavi *et al*., 2023). The liver function test (LFT) was performed to examine the hepatotoxic effect of the extracts in SD rats. This test describes the health status of the liver, including its cellular integrity, functionality, and excretion of bilirubin (Omodamiro *et al*., 2021). The results show that there was no alteration in the levels of the liver enzymes (GGT, AST, ALP, and ALT), bilirubin, total protein, and albumin between the control and test groups (Figure 3). This suggests that repeated administration of UAE ethanolic lemongrass leaves extract has no toxic effect on the liver and that could be because the extract has no bioactive principles with hepatocellular-damaging or cholestatic effects on the liver. On the other hand, it indicates the extract has a hepatoprotective function, which needs to be investigated. These results corroborate with Saenthaweesuk *et al*. (2017) who also reported that lemongrass leaf extract has no hepatotoxic effect in rats.

The kidney is highly susceptible to toxicants because of the high volume of toxins it filters from the blood which can concentrate in the tubules (Bencheikh *et al*., 2022). Alteration of kidney function leads to the accumulation of urea, creatinine, and electrolyte and acid-base imbalance (Bencheikh *et al*., 2022). Serum urea elevate as a result of impaired clearance or excess protein intake (Yusuf *et al*., 2020). Serum creatinine concentration is a function of muscle mass and activity. It's a freely excretable metabolite by the tubules of the kidney and thus elevated levels indicate diminished renal function (Kurtz & Travlos, 2018). The results from this study show that the urea, creatinine, and electrolyte concentrations were comparatively the same for all groups. However, there was a slight dose-dependent decrease in creatinine concentration (though statistically insignificant) from the control to the 1200mg/kg bw.t treatment group ($p > 0.05$) (Figure 4). Since the data show that urea concentration was almost the same for control and treatment groups, it suggests that continuous gavage of

the extract has no adverse effect on renal function. The non-significant decrease in serum creatinine concentration indicates a non-significant compromise in renal function. It suggests that the extract neither affects creatinine metabolism nor compromises tubular excretion of creatinine. The electrolyte balance, as observed in this study, is a good indicator of the normal functioning of the tubules of the kidney. The biochemical data of the kidney corroborate with the absence of histopathological alterations in the kidney sections of the rats, confirming the nontoxic effect of the UAE ethanolic lemongrass leaves extract. Our findings contradict those of (Christopher, 2018), who reported decreased glomerular filtration rate (eGFR) and creatinine clearance (CCr) in healthy volunteers. The urinalysis result corroborates the renal function results. No abnormalities were detected in the urine of both the control and treatment groups. Suggesting that repeated administration of UAE ethanolic lemongrass leaves extract has no adverse effects on renal tubular functioning. In xenobiotic monitoring, tissue-specific histopathological examination is the most extensively used method. The gross pathological and histological examination of the studied tissues revealed no extract-induced morphological, textural, or any other toxicological alteration of the studied organs in all rats (Figures 5-9). Also, no histopathological derangements such as necrosis, nuclear degeneration, cytoplasmic vacuolation, and hypertrophy were observed in the heart, liver, kidney, lung, and spleen in the control and experimental rat groups. Put together, the biochemical, haematological, physical, and pathological examination data obtained from this study confirm the safety profile of UAE ethanolic lemongrass leaves extract in SD rats. This study corroborates previous studies by (Ayenew *et al*., 2022b; Sadi & Imam, 2019).

The results from this study favourably compare with a previous study by (Ayembilla *et al*., 2023) which examined the safety profile of C. citratus leaves using the conventional Soxhlet extraction method. While the conventional Soxhlet extraction method is commonly used for the extraction of natural products, this study highlights the safety of this ultrasonic method, which is recommended for heat-sensitive compounds.

LIMITATIONS AND RECOMMENDATIONS

Only female SD rats were used for this study, which may limit the generalizability of the study's implications to males. It is recommended that future studies should assess the toxicological effects of UAE ethanolic lemongrass leaves extract on reproduction, reproductive hormones, and the development of secondary sexual characteristics to unravel the effect of the extract on sex. Besides that, the therapeutic dose and mode of action of UAE ethanolic lemongrass leaves extract should be determined. Since the study revealed that the UAE method is relatively safe, it is recommended for the extraction of heat-sensitive natural products.

CONCLUSION

Based on the data from this study, it is deduced that the UAE ethanolic lemongrass leaves extract has no deleterious effect on the body weight, haematological, and biochemical parameters of SD rats, and has no organ-specific toxicity. Thus, the UAE ethanolic lemongrass leaves extract is safe after six weeks of repeated gavage in SD rats. However, consumers should avoid higher dosages and should not completely ignore the possibility of chronic toxicity since animal studies cannot be directly extrapolated to humans.

Data Availability

All the relevant data supporting the study are included in the paper.

Declaration of competing interest

All authors declare that they have no competing interests that could affect or have the perception of affecting their objectivity or could influence or have the perception of influencing the study results or the writing of the content of the manuscript.

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