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Full Length Research

Toxicity evaluation of the fruit peel extract of *Cucumis metuliferus* in Wistar rats

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ABSTRACT: *Cucumis metuliferus* is a commonly used medicinal herb in traditional medicine. Recent investigations showed that the peel of *Cucumis metuliferus* fruit contains numerous bioactive components and remains largely unexplored in terms of its bioactive potential and safety profile. Through phytochemical screening and acute, sub-acute (28-day), and sub-chronic (90-day) toxicity evaluations in experimental models, this study sought to characterise the toxicological profile of the fruit peel extract. Phytochemical screening showed the presence of alkaloids, flavonoids, steroids, terpenoids, carbohydrates, and cardiac glycosides. According to OECD criteria, acute toxicity testing revealed no overt evidence of toxicity or fatality at dosages up to 2000 mg/kg, suggesting a significant margin of safety. Oral treatment of the extract led to significant changes in selected organ weights at 28 days and the 90-day experiment. During both research periods, most biochemical markers and haematological measures stayed within normal ranges. However, during both 28 and 90 days of treatment, there was a noticeable and statistically significant increase in the liver enzymes aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT), indicating a possible hepatic effect. These biochemical results were supported by histopathological analysis, which showed mild to moderate hepatocellular inflammation and localised necrosis in rats given dosages higher than 1000 mg/kg, with lesions more noticeable in the chronic group. These results suggest that although *C. metuliferus* fruit peel extract has minimal acute lethality, long-term usage at larger levels may be hazardous to the liver. Consequently, more research is necessary to clarify the underlying processes of the reported liver harm, and caution is recommended in its chronic therapeutic application.

Keywords: Bioactive compounds, *Cucumis metuliferus*, fruit peel, traditional medicine.

INTRODUCTION

Since ancient times, people have utilised plants for food and medicine (Evans, 2009). Nowadays, pharmacologists and researchers are diving deeper into the world of medicinal herbs to tackle a variety of health issues (Ullah *et al*., 2020; Khumalo *et al*., 2022; Mohammadi *et al*., 2022; Abdulhafiz *et al*., 2022). The World Health Organisation (WHO, 2008) reports that more than 80% of the global population relies on traditional medicine as their healthcare option, especially in low- and middle-income countries where plant-based remedies are the norm (Palhares *et al*., 2015; Kaid *et al*., 2019).

While many people believe that natural products are safer, more effective, and come with fewer side effects than synthetic drugs, that is not always the case (Aschwanden, 2001; Kaid *et al*., 2019). In fact, safety profiles often do not get the attention they deserve, leading to numerous studies that highlight serious organ toxicities, especially liver and kidney damage linked to the use of unmeasured crude plant extracts (Navarro *et al*., 2017; Xu *et al*., 2020; Joung and Son, 2024; Ibezim *et al*., 2026). This underscores the need for thorough toxicological assessments before these remedies can be safely used in treatment to avoid significant health risks.

In the realm of phytotherapy, the Cucurbitaceae family includes *Cucumis metuliferus*, better known as the African horned melon or kiwano (Lim, 2019; Vieira, 2021). This

unique fruit has caught the world's attention for its availability, affordability, and impressive array of bioactive compounds (Šeregelj *et al*., 2022). Recent studies have shown that the fruit peel, often tossed aside as agricultural waste, is actually packed with bioactive constituents with pharmacological activities such as antioxidant, antimicrobial, and immunomodulatory effects (Ezekaibeya *et al*., 2020; Šovljanski *et al*., 2022; Zhu *et al*., 2022; Šeregelj *et al*., 2022).

While previous toxicological studies on *C. metuliferus* have primarily focused on the pulp, seeds, or fruit juice (Achikanu *et al*., 2020; Ani *et al*., 2020; Sadou *et al*., 2007), the specific safety profile of the fruit peel remains largely unevaluated despite its distinct phytochemical composition. Therefore, this study was designed to evaluate the acute (single-dose administration), sub-acute (28-day) repeated-dose, and sub-chronic (90-day) repeated-dose toxicity of methanolic *C. metuliferus* fruit peel extract in Wistar rats using behavioural, haematological, biochemical, organ-weight, and histopathological parameters.

MATERIALS AND METHODS

Plant collection and extraction

Fresh ripe fruit of *Cucumis metuliferus* was collected from Jos South Local Government Area, Plateau State, Nigeria in September 2024. The collected fruits were taken to the Federal College of Forestry, Jos, Nigeria, for specimen identification and authentication, and a voucher specimen (FHJ1223) was deposited in the plant's herbarium.

Extraction procedure

The method of Anyanwu *et al*. (2014) was used with slight modifications. The ripe fruits were washed in three changes of sterile distilled water to reduce surface contaminants. The fruits were cut open with the aid of a clean knife to discard the pulp and seeds, allowing the peel to sun-dry until it is completely dried. Two hundred and fifty grams of pulverised fruit peel powder was mixed with 500 mL of 70% aqueous methanol in a sealed glass container. To ensure maximum mass transfer and active constituent dissolution, the mixture was let to sit at room temperature for 72 hours, with intermittent agitation to help the active ingredients dissolve and transfer effectively. After the maceration process, the mixture was passed through a double layer of muslin cloth and then through Whatman No. 1 filter paper to get a nice, clear liquid. The filtrate was concentrated using a rotary evaporator (IKA, Germany) at 55 °C under reduced pressure to remove the organic solvent. The percentage extraction yield was calculated to be 18.0% w/w of crude extract. Finally, the semi-solid extract was transferred into a clean, airtight amber glass container and was stored in the fridge at 4°C until use.

Determination of physical properties of the extracts

The colour of the extract was assessed visually immediately after the removal of the solvent by the rotary evaporation process. The pH was tested using a Jenway pH meter. Texture was felt manually between the fingers (Nwadiaro *et al*., 2015).

Phytochemical screening of the extracts

Phytochemical analysis was conducted following standard procedures (Sofowora, 2008; Evans, 2009).

Test for alkaloids

About 0.5 g of extract was stirred with 3 mL of 1% aqueous hydrochloric acid (HCl) on a steam bath and filtered. 1 mL of the filtrate was transferred into test tube 1 and test tube 2. To test tube 1, a few drops of Dragendorff's reagent were added; the occurrence of an orange-red precipitate was taken as positive. To test tube 2, 1 mL of Mayer's reagent was added and the appearance of a buff-colored precipitate was recorded as an indication of the presence of alkaloids.

Test for tannins

About 0.5 g of the plant extract was stirred with 1 mL of distilled water, filtered, and a few drops of ferric chloride solution were added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins.

Test for cardiac glycosides

About 100 mg of the extract was taken in a test tube. A volume of 2.5 mL of dilute H2SO4 was added and boiled in a water bath for 15 min. The resultant solution was cooled and neutralised with 20% potassium hydroxide solution. About 5 mL of a mixture of Fehling's solution A and B was added and boiled for 3 min. A brick-red precipitate indicated the hydrolysis of a reducing sugar, an indication of glycosides.

Test for flavonoids

Lead acetate test: About 5 mL of the extract was introduced into a test tube, and 3 mL of lead acetate solution was added. A yellow precipitate indicated the presence of flavonoids. **Sodium hydroxide test:** About 5 mL of 20% sodium hydroxide was added to an equal volume of extract, resulting in a yellow solution. Changes in colour from yellow to colourless upon the addition of

dilute hydrochloric acid indicated the presence of flavonoids.

Test for saponins

A weight of 0.5 g of the extract was shaken vigorously with 3 mL of distilled water in a test tube. Frothing that persisted upon warming was taken as preliminary evidence for the presence of saponins.

Test for steroids

About 100 mg of the extract was dissolved in 2 mL of chloroform, and sulfuric acid was added to form a lower layer. A reddish-brown colour at the interface is indicative of the presence of steroids.

Test for anthraquinones

Borntrager's test was conducted to detect the presence of anthraquinones. To 0.5 g of extract, 5 mL of chloroform was added and shaken for 5 min. To the filtrate, an equal volume of 10% ammonia solution was added. A pink, violet, or red colour in the lower layer indicated the presence of free anthraquinones.

Experimental animals

Forty Wistar albino rats comprising both sexes (20 males and 20 females) weighing 150–165 g were used in the repeated-dose (28- and 90-day) administrations, while 10 female non-pregnant Wistar albino rats were used in the acute (single-dose) administration study. Animals were housed in standard cages under controlled conditions (temperature 22±3°C, relative humidity of 60%, and a 12-hour light/dark photoperiod) and were fed standard pelleted feed and clean water ad libitum. All animals were handled according to the guidelines for the care and use of laboratory animals as stated by the National Research Council (2011), and institutional ethical clearance (02/02/20) was obtained before carrying out the study. The Animal Research Reporting of *In* Vivo Experiments (ARRIVE) guidelines were strictly observed (Percie du Sert *et al*., 2020).

Sample size

The sample size was determined using the resource equation method (Charan and Biswas, 2013). With four experimental groups of five animals each (totalling 20 animals), the degrees of freedom (E) for the analysis of variance was 16, which falls within the optimal range of 10 to 20 to ensure adequate statistical power while minimising animal use.

E = Total number of animals – Total number of groups

Total number of animals = No. of animals per group x No. of groups

E = (No. of groups x No. of animals per group) – No. of groups

E is the degree of freedom of ANOVA, and its value is considered scientifically adequate if it lies between 10 and 20

In this study; Number of groups = 4, Number of animals per group = 5, and Total number of animals =4 x 5

E = 20-4 = 16

Experimental design

Acute toxicity study

The Organisation for Economic Co-operation and Development (OECD) 'Up-and-Down' method described in OECD guideline 425 (2022) was used for the acute toxicity study of the methanolic extract of *C. metuliferus* fruit peel. Female non-pregnant rats were randomly divided into 2 groups of 5 rats each. Group 1 served as the control and was administered 1 mL/100 g of distilled water. Group 2 was administered an oral single limit dose of the extract at 2000 mg/kg body weight. All rats were fasted overnight from food before dosing but were allowed free access to water. Animals were observed continuously for the first 30 min after administration, every 1 hour for 4 hours, and daily thereafter for a total of 14 days. Signs of toxicity such as salivation, tremors, convulsions, diarrhoea, changes in the skin, fur, eyes, and mucous membranes, or death were noted. Body weights were recorded on days 0, 7, and 14. Weekly feed and water consumption were also measured and recorded. At the end of the experiment, blood was collected for haematological and biochemical analysis. Vital organs (liver, kidneys, lungs, heart, brain, spleen, and colon) were collected, weighed, and observed macroscopically for any changes.

Sub-acute toxicity study (28-Day Repeated-Dose Toxicity, OECD 407)

This study was conducted using the repeated-dose toxicity method as described in OECD guideline 407 (2008). Twenty adult Wistar albino rats (balanced by sex across groups) were divided into four groups of five animals each. Group I (control) received 10 ml/kg of distilled water;

groups II–IV received 250, 500, and 1000 mg/kg of *C. metuliferus* methanolic fruit peel extract, respectively. All administrations were given by oral gavage once daily for 28 consecutive days. Animals were monitored daily for clinical signs, including physiological or behavioural changes. On day 29, the animals were fasted overnight with free access to water, and then anaesthetised by exposure to 4% halothane vapour in a closed chamber. Blood was collected via cardiac puncture into EDTA-coated tubes and plain tubes for haematological and biochemical analyses, respectively, after which the animals were euthanised by cervical dislocation. Organs (brain, liver, kidneys, spleen, heart, lungs, and colon) were carefully removed, washed with distilled water, weighed, and examined macroscopically for gross changes. Harvested organs were fixed in 10% neutral buffered formalin for histopathological examination. Relative organ weights were calculated as described below (Wu *et al*., 2020):

Weight gain= Final weight of rat (g) - Initial weight of rat (g)

Relative organ weight= organ weight (g)/body weight (g) x 100

Sub-chronic toxicity study (90-Day Repeated-Dose Toxicity, OECD 408)

Twenty Wistar albino rats of both sexes were divided into four groups of five rats per group following random allocation. Group 1 served as the control and received 10 mL/kg distilled water only. Groups 2, 3, and 4 received 250, 500, and 1000 mg/kg body weight, respectively. The extract was dissolved in distilled water and administered by gavage once daily for 90 consecutive days. On day 91, the animals were fasted overnight with free access to water, anaesthetised with 4% halothane vapour, and blood was collected via cardiac puncture into EDTA and non-EDTA tubes. Vital organs (liver, kidneys, heart, lungs, spleen, brain, and colon) were collected, cleaned with saline, and weighed. Relative organ weights were calculated as described earlier. Animals were observed daily for clinical and toxicological signs throughout the study period.

Hematological parameters

Laboratory analysis was carried out using blood samples obtained to determine the effect of the extract on hematological parameters, including Packed Cell Volume (PCV), Hemoglobin (HB), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Red Blood Cells (RBC), White Blood Cells (WBC), Lymphocytes, Neutrophils, Monocytes, Eosinophils, and Platelets. A Mindray Haematology Analyser (BC-5100) was used to analyse these parameters.

Biochemical analysis

Laboratory analysis was carried out to determine the effect of the extract on biochemical parameters, including Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT) (Dashti *et al*., 2022), total cholesterol (TC), triglycerides (TG), fasting blood sugar (FBS), urea, and creatinine, measured using an automatic chemistry analyzer (COBAS C111).

Histopathological examination

Harvested organs (kidney, heart, liver, brain, colon, lungs, and spleen) were immediately fixed in 10% neutral buffered formalin and embedded in paraffin wax. Tissue sections were cut at 5 µm thickness using a microtome, dewaxed in xylene, hydrated in decreasing concentrations of alcohol, and stained with hematoxylin and eosin (H&E). The stained sections were observed under a light microscope by an expert pathologist, and photomicrographs were taken at x 400 magnification.

Statistical analysis

Data were analysed using GraphPad Prism software (version 9.0) and expressed as mean ± SEM. Data were assessed for normality prior to analysis. Comparisons among multiple groups were evaluated using one-way ANOVA followed by Tukey's post-hoc multiple comparison test. Differences were considered statistically significant when the p-value was below 0.05 ($p<0.05$).

RESULTS AND DISCUSSION

Phytochemical screening results

Phytochemical investigations of the methanolic extract of *Cucumis metuliferus* fruit peel revealed the presence of alkaloids, flavonoids, steroids, terpenoids, carbohydrates, and cardiac glycosides. Anthraquinones, tannins, and saponins were absent (Table 1). These findings are consistent with those reported by Aliero and Gumi (2012), with the exception of steroids, which were detected in the present peel extract but noted as absent in their whole-fruit analysis. The findings of this study are similar to those of Ezekaibeya *et al*. (2020). The presence of these bioactive secondary metabolites highlights the therapeutic potential of the fruit peel.

Acute toxicity studies

Acute toxicity testing aimed to determine the potential short-term toxicity of the extract. Administration of a single limit dose of 2000 mg/kg produced no treatment-related mortality, behavioural abnormalities, or overt toxic signs

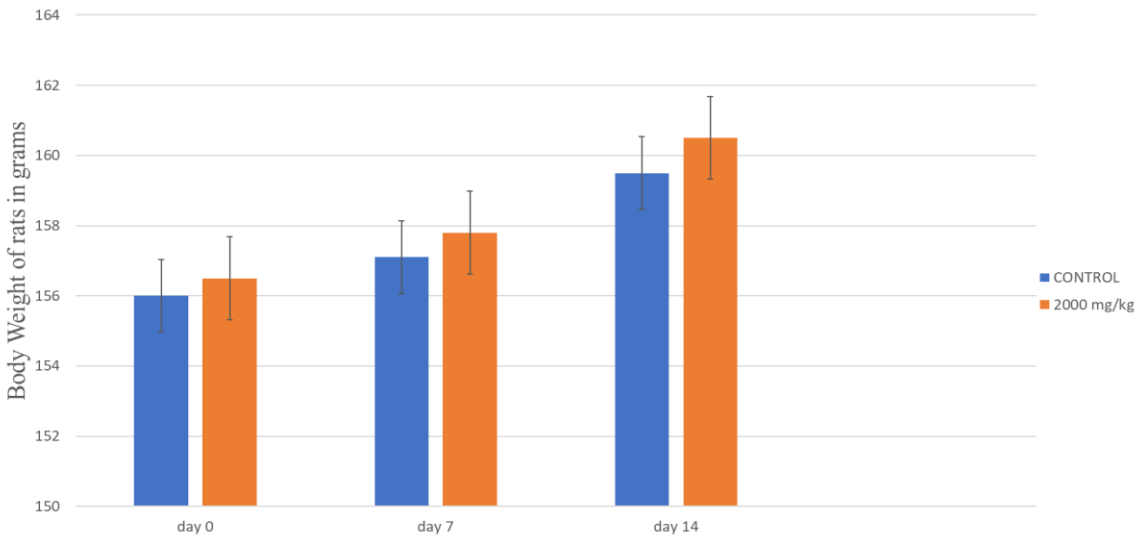

Figure 1. Body weight of rats after a single dose of *C.* metiliferus peel extract.

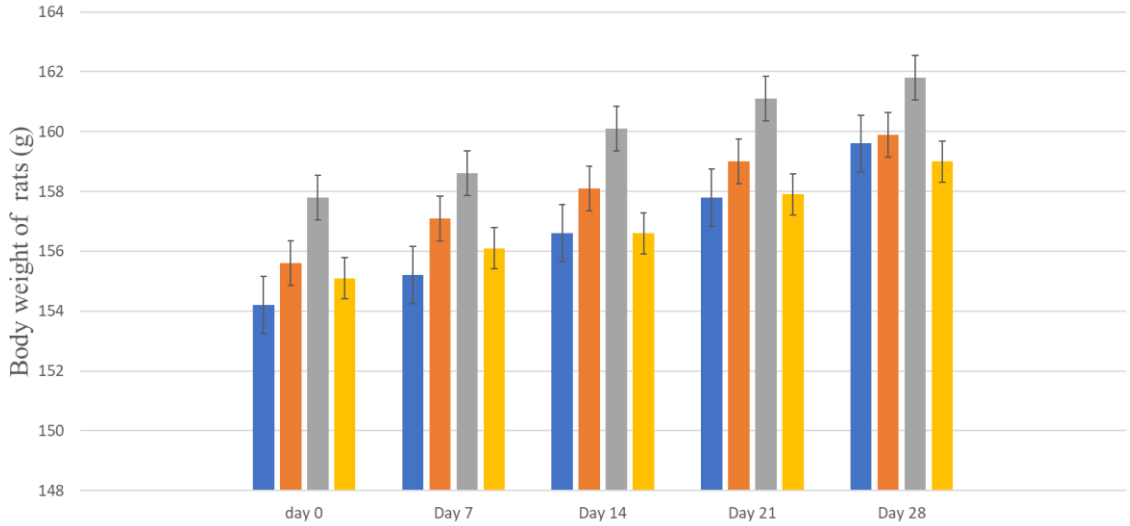

Figure 2. Body Weight of Rats Treated with *C. metiliferus* Peel Extract for 28 days.

over the 14-day observation period (as seen in Figure 1 for body weight changes alongside Figures 4 and 5 for weekly feed consumption). This indicates that the median lethal dose (LD_{50}) of the methanolic fruit peel extract is greater than 2000 mg/kg, suggesting a favourable acute safety profile, which aligns with previous evaluations of *C. metuliferus* fruit preparations (Wannang *et al*., 2008).

Body weight and feed/water consumption

Monitoring body weight and nutritional intake is essential for identifying systemic toxicity of xenobiotics (OECD, 2018). In this study, no statistically significant differences in body weight gain or weekly feed and water consumption were observed between the control and treatment groups across the acute (Figure 1, 4 and 5), 28-day sub-acute (Figure 2 for body weights, Figures 6–7 for weekly consumption). In the 90-day sub-chronic studies, there was a significant reduction in feed consumption from week 6 to week 12. while body weight and water consumption was not different from that of control (Figure 3 for body weights, Figures 8–9 for weekly consumption) at all tested dose levels (250,500 and1000 mg/kg), though Mukherjee and Mukherjee (2019), Usman *et al*. (2014) and Bamidele *et al*. (2024) reported that phytochemicals in the extract, such as alkaloids, flavonoids, and phenols may control appetite and body weight.

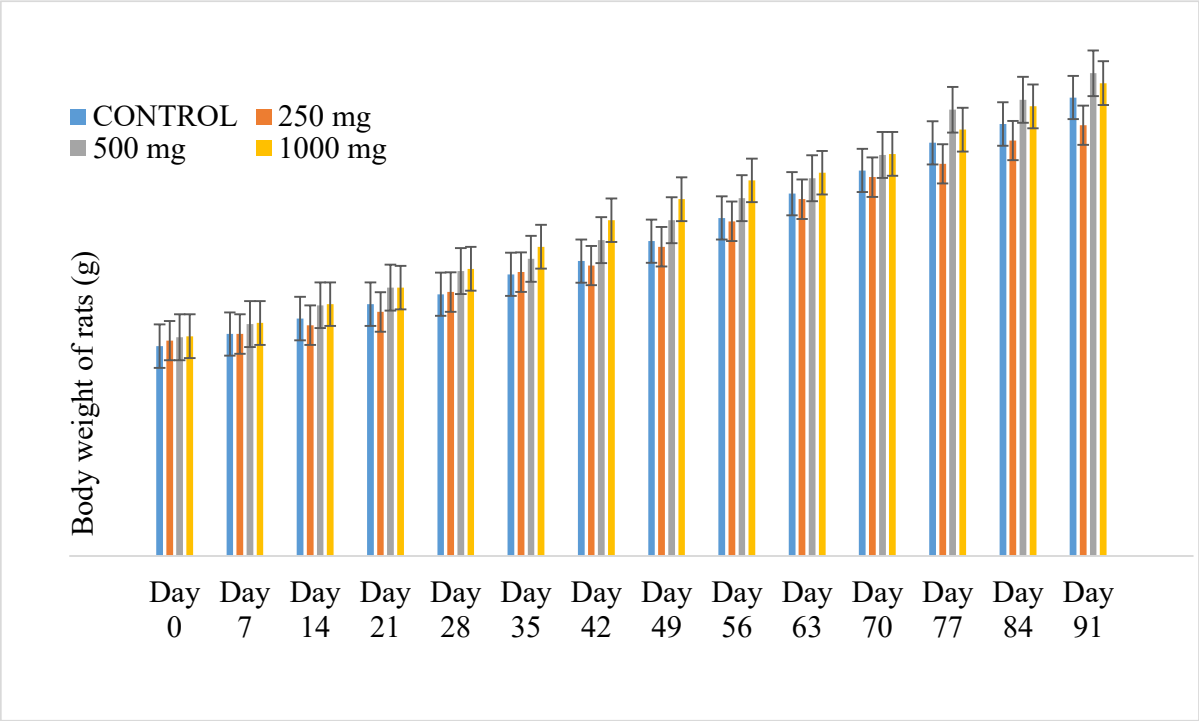

Figure 3. Body weight of rats treated with *C. metiliferus* extract for 90 days.

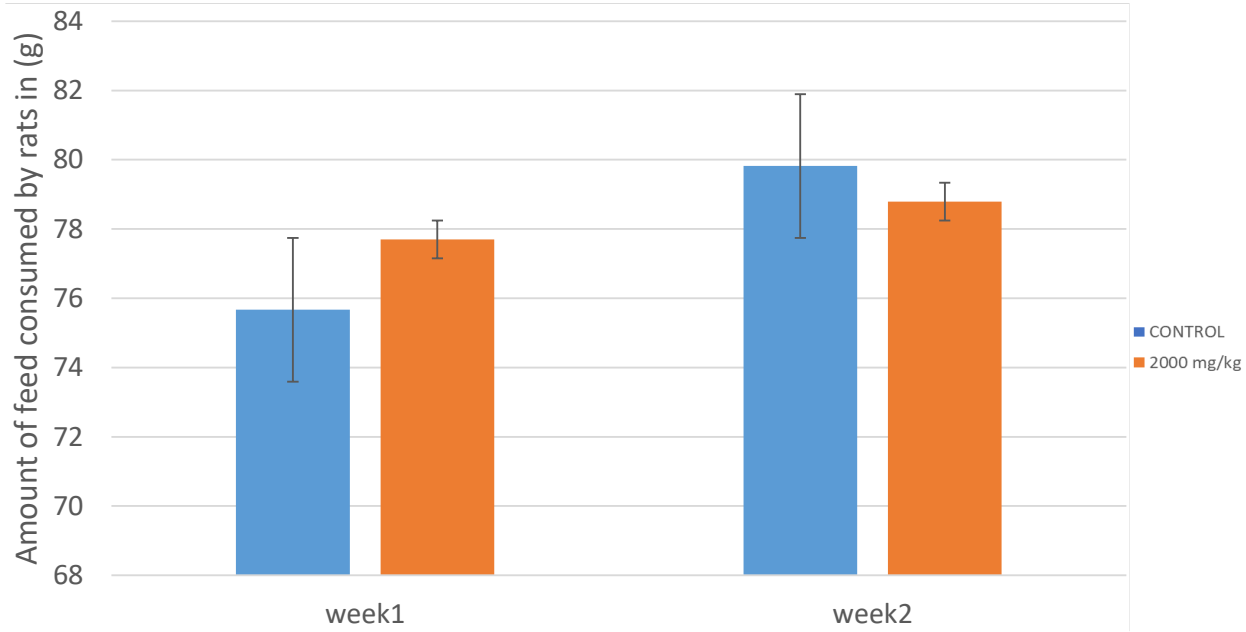

Figure 4. Weekly consumption rate of rats treated with a single dose of *C. metuliferus* peel extract.

Organ weight changes

Organ weight changes can be indicative of chemical-induced toxicity when accompanied by histological alterations.In single-dose acute administration, absolute and relative organ weights showed no significant adverse deviations (Tables 2 and 3) In the 90-day sub-chronic administration at doses of 500 mg/kg and 1000 mg/kg,

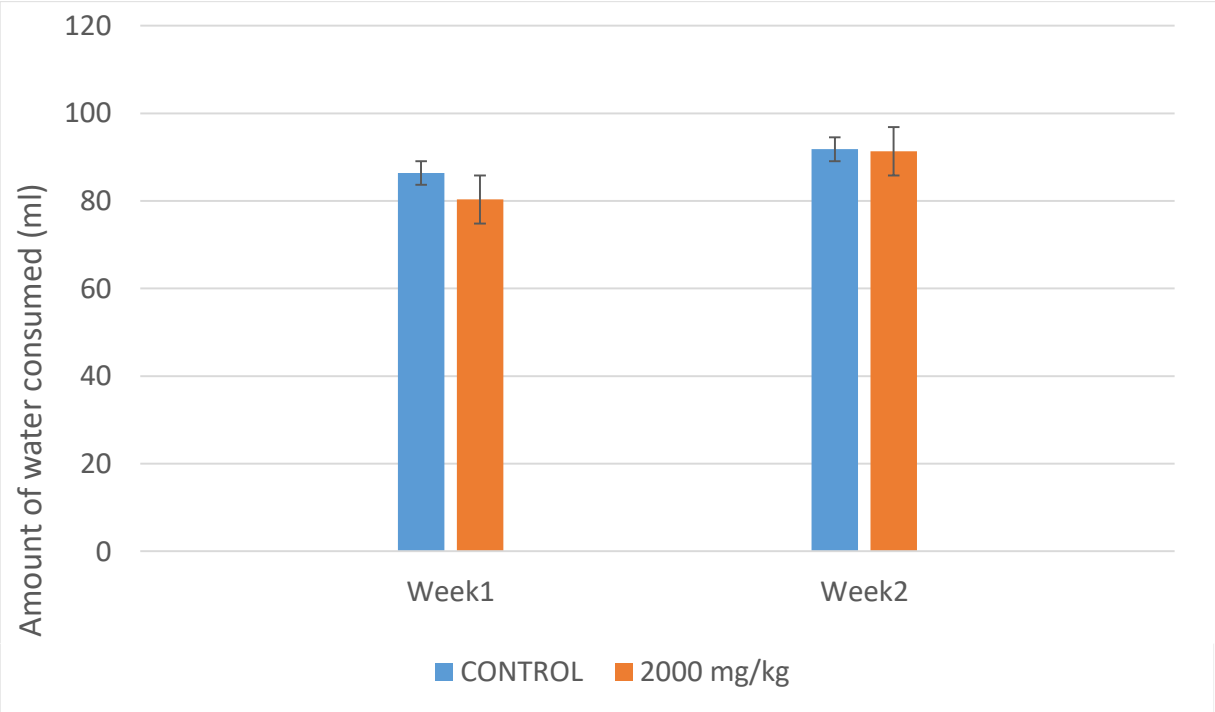

Figure 5. Weekly water consumption of rats treated with a single dose of *C. metuliferus* peel extract.

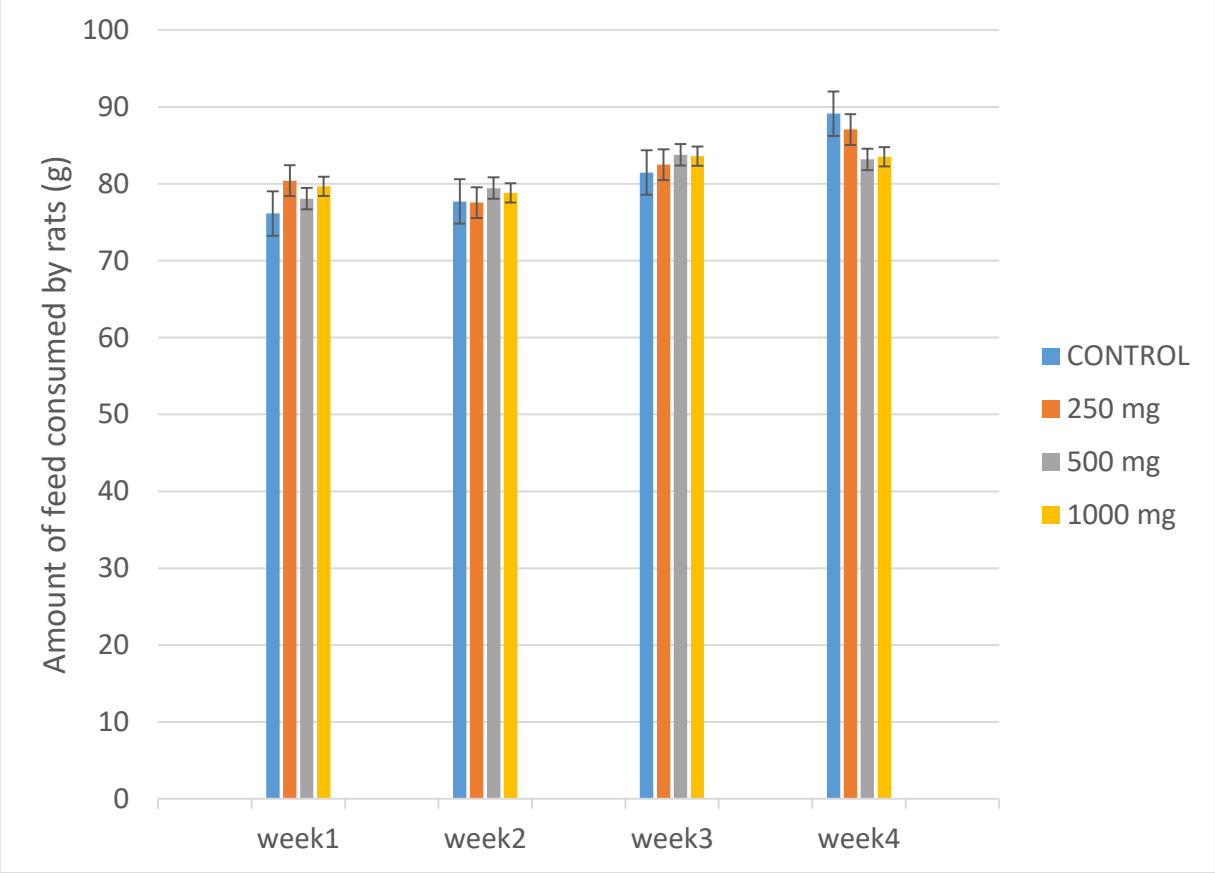

Figure 6. Weekly feed consumption of rats treated with *C. metuliferus* peel extract for 28 days.

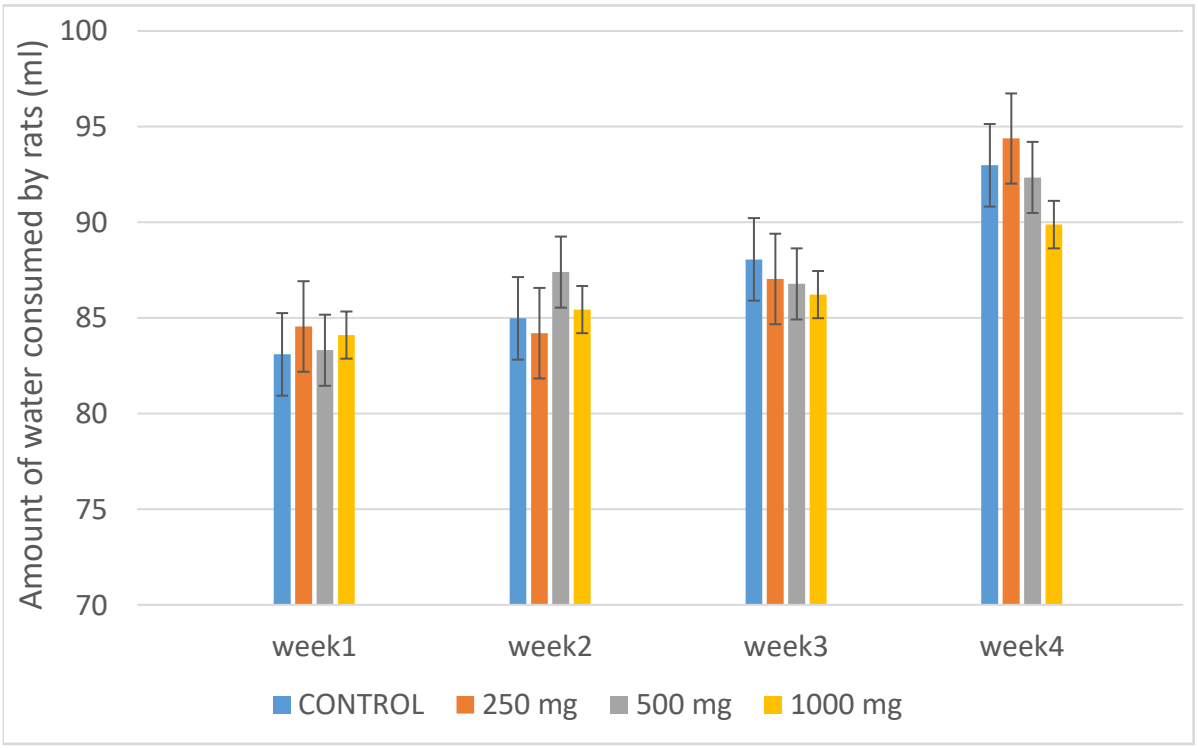

Figure 7. Weekly water consumption of rats treated with *C. metuliferus* peel extract for 28 days.

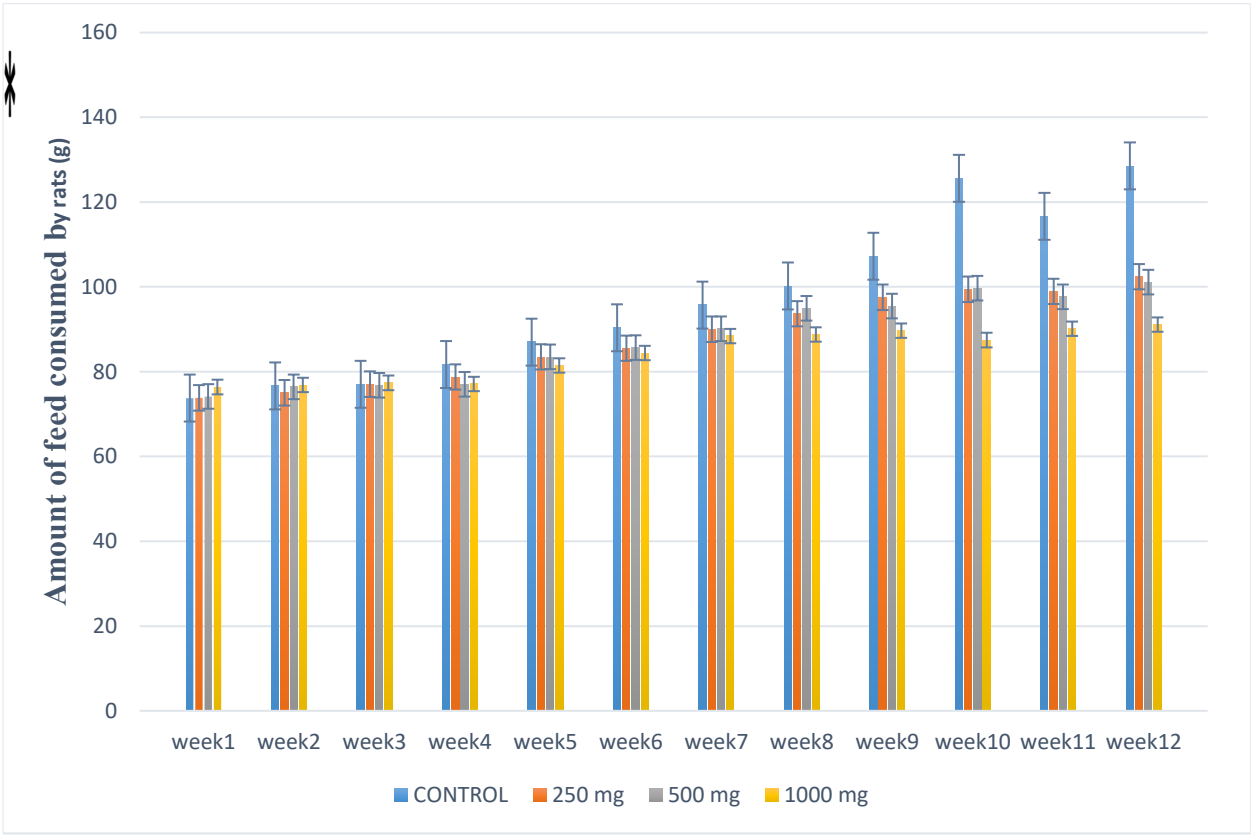

Figure 8. Weekly feed consumption by rats treated with *C. metuliferus* peel extract for 90 days.

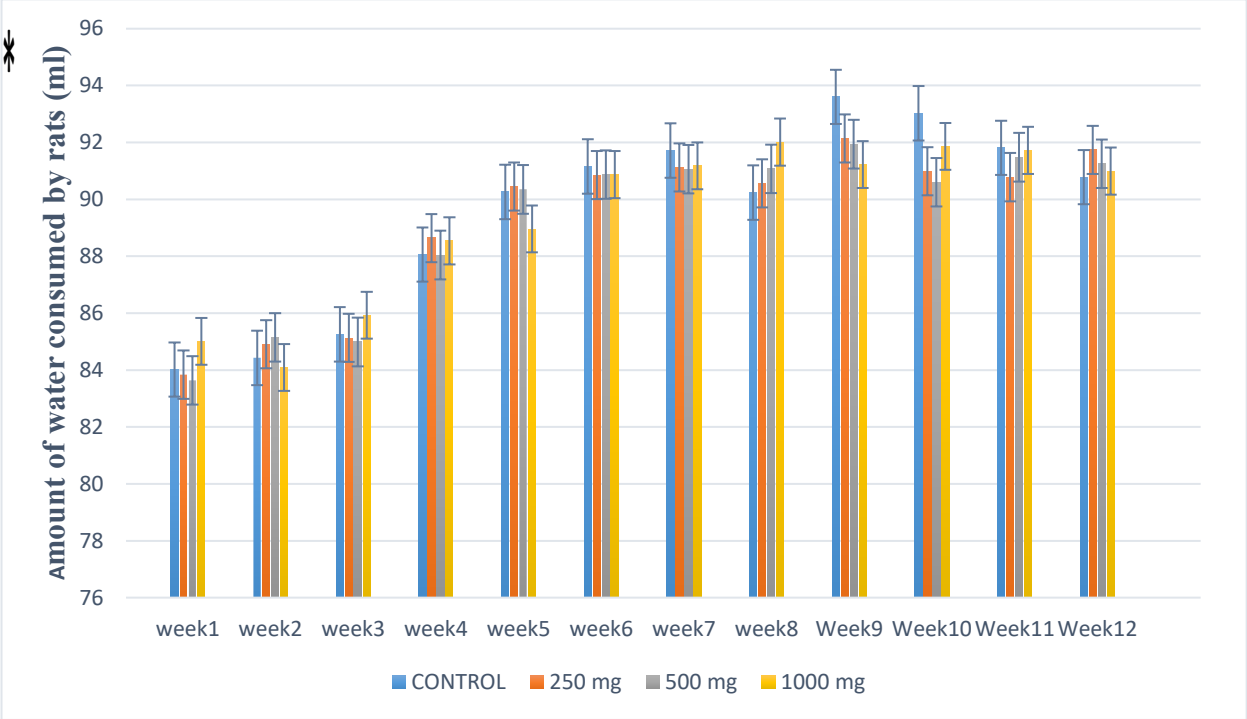

Figure 9. Weekly water consumption by rats treated with *C. metuliferus* peel extract for 90 days.

Table 1. Bioactive compounds in *Cucumis metuliferus* fruit peel.

Phytochemicals	Results
Alkaloids	+
Saponins	-
Tannins	-
Flavonoids	+
Anthraquinones	-
Steroids	+
Terpenoids	+
Carbohydrates	+
Cardiacglycoside	+

Key: - = Absent, + = Present.

Table 2. Absolute organ weight of rats treated with *C. metuliferus* peel extract in single-dose administration.

Parameter	Control	2000 mg/kg
Heart	0.124 ± 0.004	0.118 ± 0.006
Liver	4.522 ± 0.176	4.418 ± 0.162
Kidney	0.302 ± 0.004	0.302 ± 0.002
Lungs	0.302 ± 0.004	0.298 ± 0.004
Spleen	0.182 ± 0.005	0.172 ± 0.006
Brain	6.870 ± 0.030	6.818 ± 0.029
Colon	2.628 ± 0.035	2.550 ± 0.083

Data are expressed as Mean±SEM; *significant is taken at p<0.05 when the treated group is compared with the control, n=5.

Table 3. Relative organ weight of rats treated with *C. metuliferus* peel extract in single-dose administration.

Parameter	Control	2000 mg/kg
Heart	0.0780 ± 0.002	0.072 ± 0.004
Liver	3.700 ± 0.061	3.764 ± 0.067
Kidney	0.188 ± 0.002	0.190 ± 0.000
Lungs	0.186 ± 0.002	0.188 ± 0.002
Spleen	0.116 ± 0.002	0.108 ± 0.003
Brain	4.310 ± 0.021	3.916 ± 0.182
Colon	1.650 ± 0.026	1.602 ± 0.040

Data are expressed as Mean ± SEM; *significant is taken at p<0.05 when the treated group is compared with the control, n=5.

significant increases (p<0.05) in absolute and relative spleen and colon weights were observed compared to the control group (Tables 6 and 7), alongside similar relative organ weight variations observed in the 28-day study (Table 4 and 5). While alterations in organ weights are traditionally investigated as markers of toxicity, the absence of corresponding degenerative lesions in these tissues suggests that these variations may represent adaptive physiological responses rather than direct pathological damage.

Haematological and biochemical parameters

Haematological profiles remained largely unaffected

Table 4. Absolute Organ Weight of Rats Treated with C.*metuliferus* Peel Extract for 28 Days

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Heart	0.700 ± 0.021	0.724 ± 0.008	0.738± 0.004	0.692 ± 0.027
Liver	4.740 ± 0.255	4.614 ± 0.039	4.716 ± 0.080	4.670 ± 0.058
Kidney	0.920 ± 0.026	0.906 ± 0.020	0.932 ± 0.009	0.942 ± 0.010
Lungs	0.985 ± 0.038	1.004 ± 0.019	1.016 ± 0.024	0.996 ± 0.019
Spleen	0.455 ± 0.038	0.506 ± 0.019	0.404 ± 0.090	0.472 ± 0.036
Brain	6.830 ± 0.008	6.750 ± 0.082	6.774 ± 0.034	6.616 ± 0.067
Colon	2.638 ± 0.023	2.624 ± 0.031	2.630 ± 0.041	2.636 ± 0.030

Data are expressed as Mean ± SEM; *significant at p< 0.05 when the treated group is compared with the control, n=5.

Table 5. Relative organ weight of rats treated with *C. metuliferus* peel extract for 28 days.

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Heart	0.437 ± 0.010	0.450 ± 0.004	0.458 ± 0.004	0.436 ± 0.013
Liver	2.97 ± 0.152	2.886 ± 0.021	2.914 ± 0.052	2.900 ± 0.020
Kidney	0.578 ± 0.018	0.586 ± 0.017	0.576 ± 0.008	0.594 ± 0.007
Lungs	0.618 ± 0.019	0.626 ± 0.013	0.626 ± 0.017	0.626 ± 0.012
Spleen	0.318 ± 0.010	0.320 ± 0.011	0.306 ± 0.010	0.294 ± 0.021
Brain	4.280 ± 0.004	4.296 ± 0.058	4.146 ± 0.021	4.160 ± 0.039
Colon	1.653 ± 0.011	2.624 ± 0.031*	2.630 ± 0.041*	2.636 ± 0.030*

Data are expressed as Mean ± SEM; *significant is taken at p<0.05 when the treated group is compared with the control, n=5.

Table 6. Absolute organ weight of rats treated with *C. metuliferus* peel extract for 90 Days.

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Heart	0.712 ± 0.017	0.688 ± 0.028	0.728 ± 0.008	0.680 ± 0.024
Liver	4.646 ± 0.031	4.486 ± 0.038	4.672 ± 0.043	4.712 ± 0.100
Kidney	0.952 ± 0.011	0.912 ± 0.029	0.950 ± 0.014	0.950 ± 0.014
Lungs	0.994 ± 0.011	0.972 ± 0.021	0.978 ± 0.012	0.984 ± 0.019
Spleen	0.506 ± 0.026	0.534 ± 0.011	0.554 ± 0.037	0.588 ± 0.015
Brain	6.886 ± 0.019	6.762 ± 0.129	6.586 ± 0.109	6.614 ± 0.111
Colon	2.634 ± 0.037	2.606 ± 0.047	2.874 ± 0.036*	2.882 ± 0.068*

Data are expressed as Mean ± SEM; *significant at p<0.05 when the treated group is compared with the control, n=5.

Table 7. Relative organ weight of rats treated with *C. bmetuliferus* peel extract for 90 days.

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Heart	0.390 ± 0.009	0.382 ± 0.016	0.396 ± 0.005	0.380 ± 0.008
Liver	2.648 ± 0.084	2.532 ± 0.020	2.544 ± 0.016	2.620 ± 0.015
Kidney	0.446 ± 0.046	0.518 ± 0.012	0.514 ± 0.007	0.530 ± 0.008
Lungs	0.546 ± 0.005	0.544 ± 0.009	0.526 ± 0.007	0.544 ± 0.004
Spleen	0.280 ± 0.013	0.300 ± 0.004	0.290 ± 0.014	0.3320 ± 0.006*
Brain	3.796 ± 0.019	3.814 ± 0.086	3.786 ± 0.051	3.794 ± 0.044
Colon	1.446 ± 0.017	1.470 ± 0.020	1.586 ± 0.006*	1.556 ± 0.022*

Data are expressed as Mean ± SEM; *significant at p<0.05 when the treated group is compared with the control, n=5.

across treatments, as can be seen in single administration (Table 8), except for selective elevations in red blood cell (RBC) counts and hematocrit (HCT) values at higher doses during the 28-day and 90-day administrations (Tables 9 and 10). These findings are consistent with Usman *et al*. (2018), who noted similar increases following

Table 8. Haematological profile of rats treated with *C. metuliferus* peel extract in single dose administration.

Parameter	Control	2000 mg/kg
WBCx10^9/L	92.88 ± 1.080	93.84 ± 1.280
LYMPx10^9/L	64.94 ± 0.406	64.29 ± 0.684
MONx10^9/L	0.784 ± 0.039	0.810 ± 0.033
NEUx10^9/L	3.786 ± 0.264	3.600 ± 0.384
BASOPHIL	0.342 ± 0.067	0.300 ± 0.055
EOSINOPHIL	1.428 ± 0.226	1.600 ± 0.340
RBCx10^6/µL	7.334 ± 0.012	7.330 ± 0.013
MCVx10^9/L	55.74 ± 0.223	55.83 ± 0.395
HCT (%)	43.46 ± 0.560	43.37 ± 0.389
MCHx10^9/L	24.00 ± 0.354	24.31 ± 0.340
MCHC(G/dl)	31.69 ± 0.615	32.13 ± 0.549
RWD	16.26 ± 0.046	16.33 ± 0.025
HGB(g/dl)	36.19 ± 2.182	36.52 ± 1.945
PLTx10^9/L	818.0 ± 24.98	796.0 ± 25.890

Data are expressed as Mean±SEM; *significant is taken at p<0.05 when the treated group is compared with the control, n=5. **Keys:** WBC = White Blood Cell, LYMP = Lymphocyte, MON = Monocyte, NEU = Neutrophil, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Haematocrit, HGB = Haemoglobin.

Table 9. Haematological profile of rats treated with *C. metuliferus* peel extract for 28 days.

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
WBCx10^9/L	6.942 ± 0.225	7.216 ± 0.347	7.290 ± 0.340	9.462 ± 0.178*
LYMPx10^9/L	65.31 ± 1.554	58.45 ± 5.240	55.99 ± 6.183	60.45 ± 6.381
MONx10^9/L	0.718 ± 0.065	0.646 ± 0.073	0.860 ± 0.061	0.874 ± 0.122
NEUx10^9/L	6.494 ± 0.095	6.624 ± 0.124	6.544 ± 0.591	6.296 ± 0.257
BASOPHIL	1.398 ± 0.085	1.294 ± 0.065	1.064 ± 0.129	1.212 ± 0.056
EOSINOPHIL	1.462 ± 0.403	1.476 ± 0.209	1.660 ± 0.288	1.478 ± 0.412
RBCx10^6/**µ**L	4.222 ± 0.322	3.932 ± 0.393	6.582 ± 0.123*	6.000 ± 0.283*
MCVx10^9/L	57.81 ± 5.101	58.86 ± 6.473	51.27 ± 4.909	50.78 ± 2.817
HCT (%)	46.68 ± 1.106	46.10 ± 3.032	52.58 ± 2.541	49.36 ± 2.504
MCHx10^9/L	28.24 ± 1.352	31.20 ± 2.338	27.65 ± 2.812	25.81 ± 2.065
MCHC (g/dl)	32.64 ± 0.971	31.61 ± 0.507	32.32 ± 0.798	32.20 ± 0.623
RWD	35.25 ± 0.583	35.68 ± 0.512	36.89 ± 0.688	35.13 ± 1.916
HGB(g/dl)	12.40 ± 0.349	12.10 ± 0.529	12.21 ± 0.646	12.37 ± 0.626
PLTx10^{-9}/L	125.0 ± 7.071	122.2 ± 7.546	122.8 ± 9.977	117.2 ± 6.119

Data are expressed as Mean±SEM; *Significant is taken at p<0.05 when the treated group is compared with the control, n=5. **Keys:** WBC = White Blood cell, LYMP = Lymphocyte, MON = Monocyte, NEU = Neutrophil, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = haematocrit, HGB = Haemoglobin.

C. metuliferus administration. Such changes may relate to the micronutrient and mineral content reported in *Cucumis* species (Sadou *et al*., 2007; Šeregelj *et al*., 2022), though direct erythropoietic mechanisms require further verification.

Significant biochemical alterations were confined primarily to hepatic and renal markers at the highest dose tested (1000 mg/kg). Acute single-dose administration did not show any biochemical changes compared to the control (Table 11). While in the 28-day study, serum ALT, AST, and ALP levels were significantly higher compared to the control (Table 12). Similarly, in the 90-day study; elevated serum AST, ALT, ALP, urea, and potassium concentrations were observed in the 1000 mg/kg group (p<0.05) (Table 13). These enzyme elevations strongly point towards functional hepatic stress or hepatocellular injury upon prolonged high-dose exposure, corroborating earlier findings by Wannang *et al*. (2007).

Table10. Hematological profile of rats treated with *C. metuliferus* peel extract for 90 days.

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
WBCx10^9/L	7.510 ± 0.453	7.543 ± 0.353	8.615 ± 0.515	8.845 ± 0.477
LYMPx10^9/L	75.34 ± 4.122	71.98 ± 1.843	74.56 ± 3.301	77.42 ± 5.278
MONx10^9/L	1.370 ± 0.172	1.236 ± 0.223	1.062 ± 0.148	1.098 ± 0.208
NEUx10^9/L	20.14 ± 0.493	20.29 ± 0.756	20.38 ± 1.339	19.04 ± 0.428
BASOPHIL	1.613 ± 0.254	1.518 ± 0.249	1.598 ± 0.154	1.258 ± 0.029
EOSINOPHIL	1.118 ± 0.116	1.020 ± 0.199	0.948 ± 0.184	1.120 ± 0.163
RBCx10^6/µL	7.394 ± 0.299	7.154 ± 0.422	9.133 ± 0.405*	8.986 ± 0.461*
MCVx10^9/L	50.17 ± 1.507	50.54 ± 1.382	49.70 ± 1.510	50.56 ± 2.491
HCT (%)	39.86 ± 0.466	40.12 ± 1.510	38.61 ± 2.144	50.84 ± 2.454*
MCHx10^9/L	17.34 ± 0.450	15.91 ± 1.199	16.73 ± 0.801	16.91 ± 0.618
MCHC(G/dl)	33.38 ± 0.626	33.00 ± 0.979	33.85 ± 1.093	32.91 ± 1.267
RWD	11.14 ± 0.552	11.01 ± 0.474	11.07 ± 0.718	11.72 ± 1.074
HGB(g/dl)	14.34 ± 0.481	14.05 ± 0.369	14.43 ± 0.322	14.04 ± 0.579
PLTx10^9/L	815.2 ± 33.00	805.8 ± 13.44	815.0 ± 44.76	761.4 ± 58.02

Data are expressed as Mean±SEM; *significant is taken at p<0.05 when the treated group is compared with the control, n=5. **Keys:** WBC (White Blood Cell, LYMP = Lymphocyte, MON = Monocyte), NEU = Neutrophil, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, HCT = Haematocrit, HGB = Haemoglobin.

Table 11. Some biochemical parameters of rats treated with *C. metuliferus* peel extract in single-dose administration

Parameter	Control	2000 mg/kg
FBS (mmol/L)	118.10± 0.930	117.30 ± 1.315
Na^+ (mmol/L)	147.40 ± 0.613	147.30 ± 0.806
$k^{+(}$mmol/L)	5.95 ± 0.158	5.83 ± 0.189
CL^- (mmol/L)	115.00 ± 0.692	115.70 ± 1.044
Cr (mmol/L)	0.48 ± 0.078	0.60 ± 0.041
Urea (mmol/L)	16.26 ± 0.608	16.14 ± 0.733
AST (U/l)	77.18 ± 0.597	74.70 ± 1.096*
ALT U/l)	41.99 ± 0.628	41.87 ± 0.620
ALP (U/l)	139.80 ± 1.649	139.90 ± 0.934

Data are expressed as Mean±SEM; *signifcant is taken at p<0.05 when treated group is compared with the control, n=5. **Key:** FBS = Fasting blood sugar, AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase, Cr = Creatinine.

Table 12. Some biochemical parameters of rats treated with *C. metuliferus* peel extract for 28 days.

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
FBS (mmol/L)	154.0 ± 2.318	126.6 ± 7.005*	118.0 ± 1.919*	118.0 ± 2.056*
Na^+ (mmol/L)	184.0 ± 4.818	155.3 ± 4.480*	133.5 ± 6.252*	136.7 ± 2.645*
$k^{+(}$mmol/L)	5.280 ± 0.131	5.360 ± 0.175	6.200 ± 0.095	5.808 ± 0.622
CL^- (mmol/L)	128.3 ± 3.225	103.4 ± 3.232*	98.06 ± 1.293*	104.4 ± 4.835*
Cr (mmol/L)	0.578 ± 0.066	0.455 ± 0.062	0.434 ± 0.059	0.762 ± 0.011
Urea (mmol/L)	18.61 ± 0.516	17.26 ± 0.623	18.01 ± 1.229	27.06 ± 0.752*
AST (U/l)	81.11 ± 2.683	77.22 ± 1.077	76.97 ± 1.517	110.2 ± 7.012*
ALT U/l)	52.31 ± 3.064	44.76 ± 1.364	47.78 ± 1.295	91.84 ± 13.35*
ALP (U/l)	152.2 ± 5.393	144.9 ± 3.765	139.6 ± 3.124	186.7 ± 4.333*

Data are expressed as Mean ± SEM; number of rats per group, n = 5, *significant p-value when control is compared with the test groups. **Key:** FBS = Fasting blood sugar, AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase, Cr= Creatinine.

Table 13. Some biochemical parameters of rats treated with *C. metuliferus* peel extract for 90 days.

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
FBS (mmol/L)	159.0 ± 3.130	151.8 ± 5.722	113.4 ± 3.79	115.8 ± 4.283
Na^+ (mmol/L)	150.6 ± 1.503	144.8 ± 3.760	141.6 ± 2.977	138.0 ± 4.919
$k^{+(}$mmol/L)	4.660 ± 0.268	5.320 ± 0.282	5.160 ± 0.294	6.920 ± 0.206*
CL^- (mmol/L)	104.4 ± 1.631	99.40 ± 1.208*	99.20 ± 1.158*	98.80 ± 1.020*
Cr (mmol/L)	0.442 ± 0.033	0.428 ± 0.065	0.410 ± 0.048	0.730 ± 0.033*
Urea (mmol/L)	20.46 ± 1.957	16.44 ± 0.312	17.91 ± 0.846	28.76 ± 1.768*
AST (U/l)	78.68 ± 0.599	77.34 ± 0.698	76.18 ± 0.932	104.4 ± 1.742
ALT U/l)	44.90 ± 2.237	42.69 ± 0.879	42.02 ± 0.639	82.2 ± 10.74*
ALP (U/l)	138.5 ± 2.601	140.0 ± 1.701	141.6 ± 1.979	172.5 ± 7.760*

Data are expressed as Mean ± SEM; number of rats per group, n = 5, *significant p-value when control is compared with the test groups. FBS = Fasting blood sugar, AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase, Cr = Creatinine.

Table 14. Lipid profile of rats treated with *C. metuliferus* peel extract in single dose administration.

Parameter	Control (Dw 1ml)	E.2000 (mg/kg)
TC (mg/dl)	231.6 ± 6.93	236.2 ± 5.29
TG (mg/dl)	235.6 ± 18.73	234.4 ± 17.45
HDL (mg/dl)	56.6 ± 6.27	65.1 ± 2.59
VLDL (mg/dl)	47.1 ±3.76	46.9 ± 3.49
LDL (mg/dl)	123.7 ±5.89	123.7 ± 4.062
TC/HDL (mg/dl)	3.828 ±0.21	3.650 ± 0.10

Data are expressed as Mean ± SEM; number of rats per group, n = 5, *significant p-value when control is compared with the test groups. **Key:** TC Total Cholesterol, TG = Triglyceride, HDL = High Density Lipoprotein, VLDL = Very Low Density Lipoprotein, LDL = Low Density Lipoprotein.

Table 15. Lipid profile of rats treated with *C. metuliferus* peel extract for 28 days.

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
TC (mg/dl)	208.8 ± 3.129	150.2 ± 2.608*	148.6 ± 2.198*	145.5 ± 2.314*
TG (mg/dl)	149.8 ± 2.497	120.1 ± 1.349*	121.3 ± 1.902*	123.6 ± 1.811*
HDL (mg/dl)	58.36 ± 0.260	54.28 ± 2.357	67.82 ± 3.003*	65.04 ± 2.812
VLDL (mg/dl)	29.96 ± 0.491	24.08 ± 0.317*	24.24 ± 0.382*	24.70 ± 0.359*
LDL (mg/dl)	120.3 ± 2.700	71.82 ± 4.085*	56.66 ± 4.418*	56.20 ± 3.232*
TC/HDL (mg/dl)	3.540 ± 0.040	2.82 ± 0.136*	2.160 ± 0.144*	2.160 ± 0.157*

Data are expressed as Mean ± SEM; number of rats per group, n = 5, *significant p-value when control is compared with the test groups. **Key:** TC = Total Cholesterol, TG = Triglyceride, HDL = High Density Lipoprotein, VLDL = Very Low Density Lipoprotein, LDL = Low Density Lipoprotein.

Lipid profile

The extract induced significant reductions in total cholesterol (TC), triglycerides (TG), very-low-density lipoproteins (VLDL), and low-density lipoproteins (LDL), alongside increases in high-density lipoprotein (HDL) across repeated-dose groups (Tables 14,15 and 16). Though the study by Mwangi *et al*. (2026) demonstrated that *Cucumis metuliferus* fruit extract possesses hypolipidemic properties, these lipid-modulating changes indicate an influence on lipid metabolism; they are interpreted strictly as experimental observations rather than established therapeutic benefits within the context of this safety evaluation.

Histopathological findings

Histopathological examination confirmed structural integrity across most organs at 250 mg/kg and 500 mg/kg

Table 16. Lipid profile of rats treated with *C. metuliferus* peel extract for 90 days.

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
TC (mg/dl)	167.2 ± 1.452	149.9 ± 2.96*	146.2 ± 4.76*	145.4 ± 6.640*
TG (mg/dl)	146.2 ± 1.777	123.1 ± 2.094*	125.8 ± 1.500*	126.4 ± 1.531*
HDL (mg/dl)	57.32 ± 0.802	57.34 ± 0.644	65.16 ± 1.866*	65.90 ± 1.592*
VLDL (mg/dl)	29.24 ± 0.3501	24.60 ± 0.409*	25.14 ± 0.301*	25.26 ± 0.308*
LDL (mg/dl)	80.82 ± 1.655	66.32 ± 3.596	55.86 ± 6.427*	54.50 ± 6.968*
TC/HDL (mg/dl)	2.90 ± 0.045	2.580 ± 0.037	2.28 ± 0.153*	2.200 ± 0.095*

Data are expressed as Mean ± SEM; number of rats per group, n = 5, *significant p-value when control is compared with the test groups. TC = Total Cholesterol, TG = Triglyceride, HDL = High Density Lipoprotein, VLDL = Very Low Density Lipoprotein, LDL = Low Density Lipoprotein.

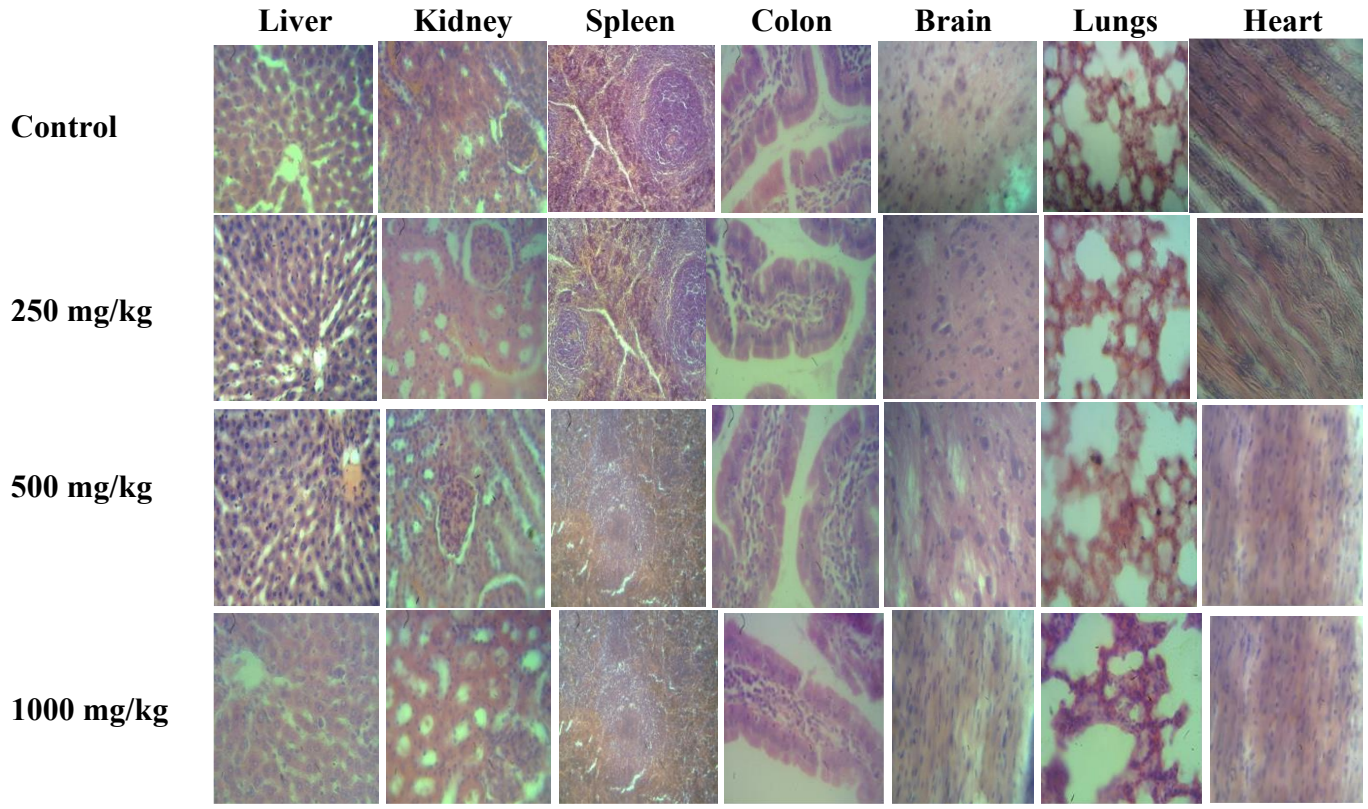

Plate 1. Histopathological findings of the liver, kidney, spleen, colon, brain, lungs and heart stained with hematoxylin and eosin (H & E x 400) in sub-chronic study.

(Plates 1 and 3). However, at the 1000 mg/kg dose level in both the 28-day and 90-day studies, marked hepatic and renal histopathological lesions were evident (Plates 2 and 4). The liver sections showed mild-to-moderate hepatocellular degeneration, necrosis, sinusoidal congestion, and inflammatory cell infiltration (Plates 2B and 4B). Renal sections revealed glomerular distortion with loss of Bowman's capsule definition and diffuse tubular necrosis (Plates 2D and 4D). These histological lesions closely parallel the significant elevation in transaminases and renal function markers, confirming target-organ toxicity at the 1000 mg/kg threshold. This finding is similar to that of Jimam *et al*. (2011), which showed that at a dose of 1000 mg/kg of *Cucumis metuliferus* fruit extract, liver tissue necrosis and degeneration occurred, whereas the kidneys showed renal epithelial cell damage.

Study limitations

This study provides a comprehensive toxicological dataset but has certain limitations, including the use of a single extraction solvent (70% methanol), a relatively small

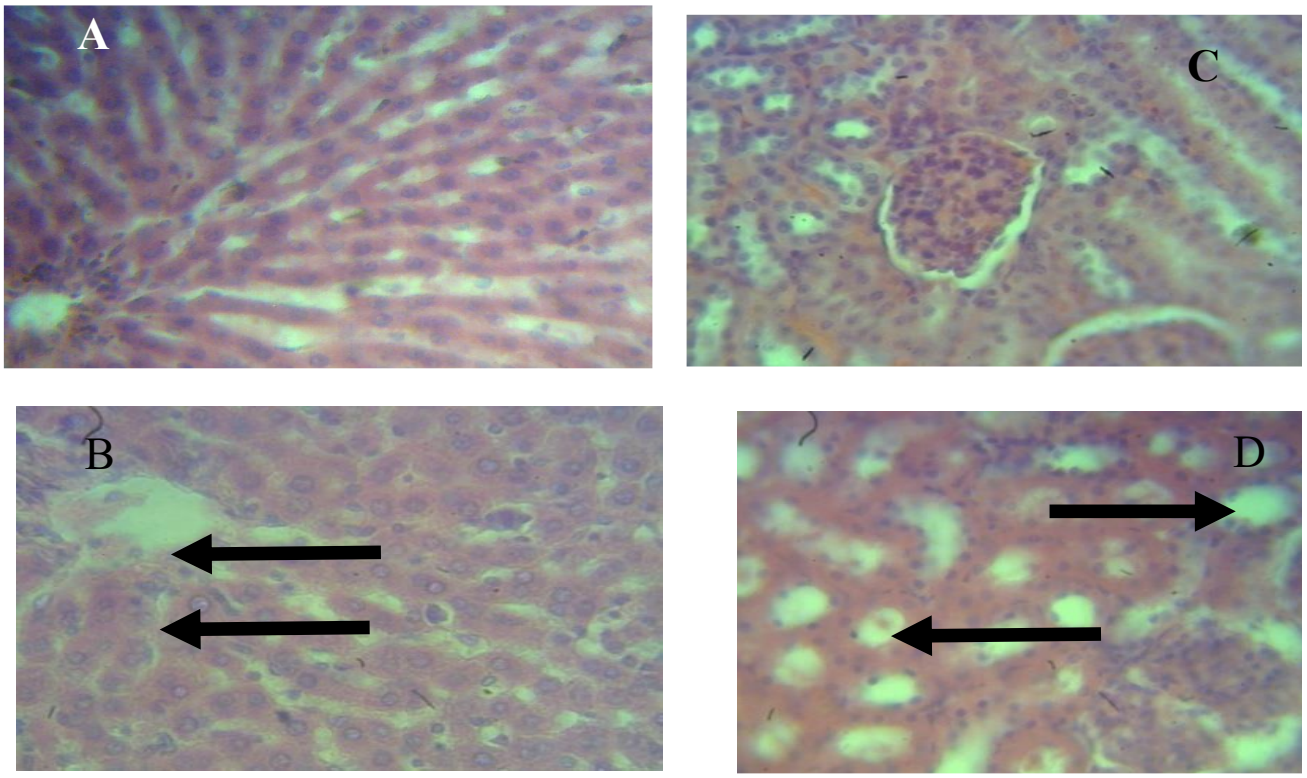

Plate 2. Histogram of liver and kidney stained with hematoxylin and eosin (H & E x 400) in sub-chronic study. **A.** Histological section of liver of the control rat (H&E x400) in sub-acute administration. Section showing normal hepatocytes. **B.** Histological section of test rat liver at a dose of 1000mg/kg (H&E x400). Showing severe degeneration, necrosis and mild to moderate inflammatory reaction in extract-treated animals. **C.** Histological section of kidney of control rat (H&E x400). Section showing normal histological features including glomerulus and tubules. **D.** Histological section of test rat kidney at dose of 1000 mg/kg (H& E x400). Section showing a glomerulus with loss of surrounding Bowman's capsule, diffuse tubular necrosis.

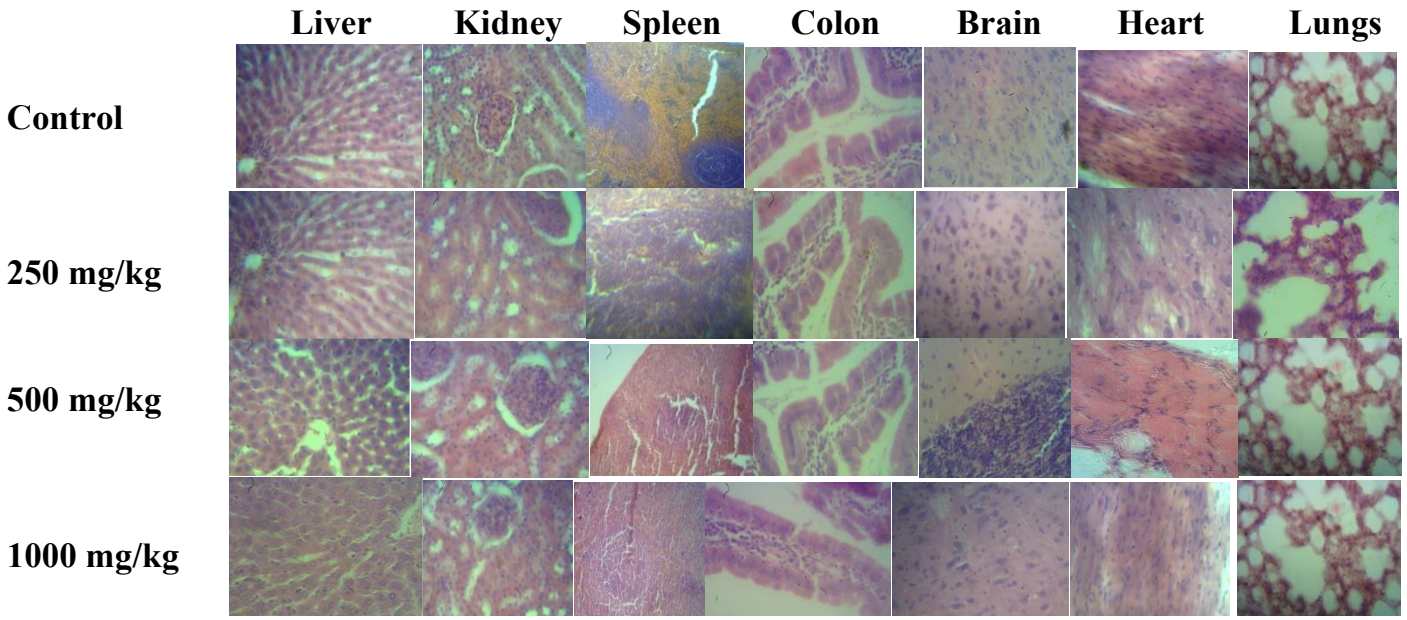

Plate 3. Histopathological findings of the liver, kidney, spleen, colon, brain lungs and heart stained with hematoxylin and eosin (H & E x 400) in the sub-chronic study [There were no overt pathological lesions at dose levels (250mg and 500mg) studied. However, at doses ≥ 1000 mg/kg, histopathological changes including inflammation and necrosis were observed in both kidney and liver tissues].

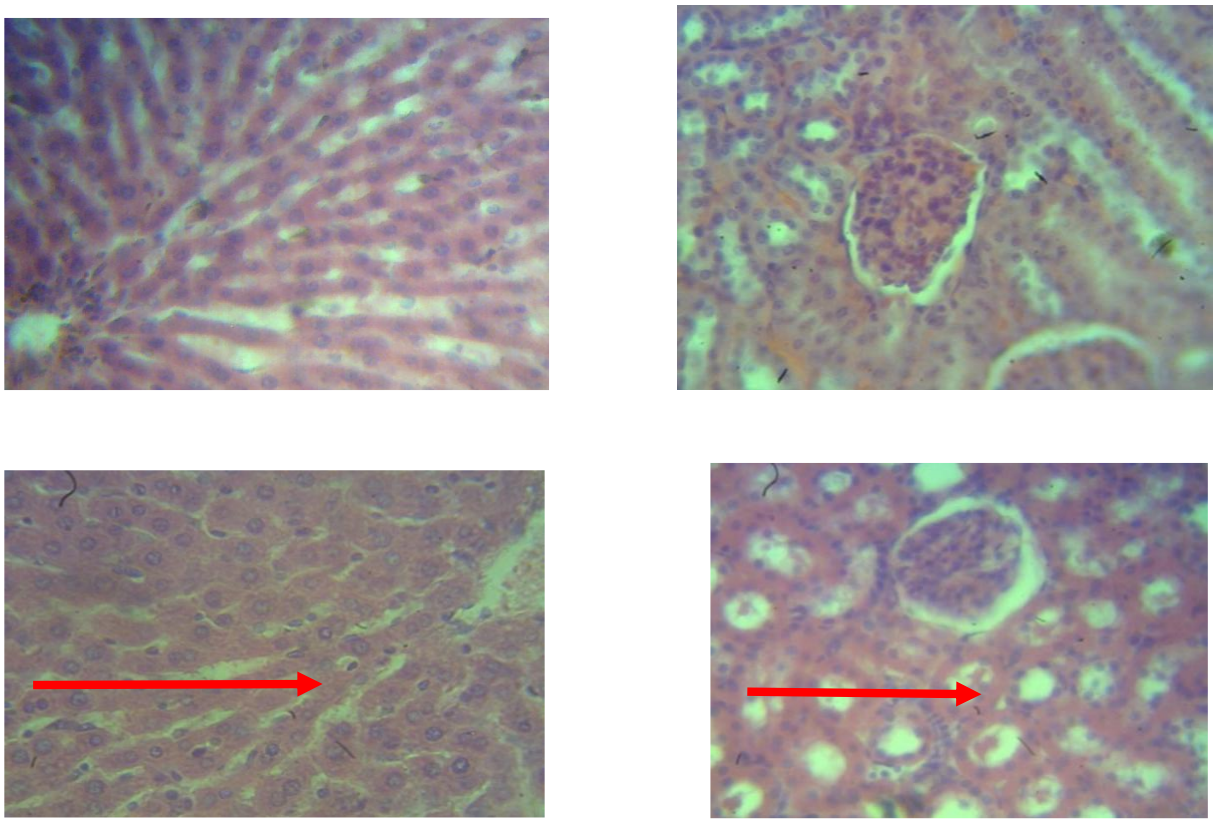

Plate 4. Histogram of liver and kidney stained with hematoxylin and eosin (H & E x 400) in sub- chronic study. **A.** Histological section of liver of the control rat (H&E x400) in sub-chronic administration. Section showing normal hepatocytes. **B.** Histological section of test rat liver at a dose of 1000mg/kg (H&E x400). Showing severe degeneration, necrosis and mild to moderate inflammatory reaction in extract-treated animals. **C.** Histological section of kidney of control rat (H&E x400). Section showing normal histological features including glomerulus and tubules. **D.** Histological section of test rat kidney at dose of 1000 mg/kg (H& E x400). Section showing a glomerulus with loss of surrounding Bowman's capsule, diffuse tubular necrosis]

sample size per group (n = 5), and the absence of a recovery group to test the reversibility of the observed hepatic and renal lesions. Based on the experimental outcomes, the No-Observed-Adverse-Effect Level (NOAEL) for the methanolic fruit peel extract is established at 500 mg/kg/day, while the Lowest-Observed-Adverse-Effect Level (LOAEL) is established at 1000 mg/kg/day based on hepatic and renal biochemical and histopathological endpoints.

Conclusion

This study evaluated the acute, 28-day sub-acute, and 90-day sub-chronic toxicity of the methanolic extract of *Cucumis metuliferus* fruit peel in Wistar rats. The findings indicate that the extract is well-tolerated at acute doses and repeated low-to-moderate doses (250-500 mg/kg). However, prolonged daily administration at 1000 mg/kg results in significant biochemical and histopathological evidence of hepatotoxicity and nephrotoxicity.

Recommendation

Caution should be exercised regarding the chronic, unmonitored consumption of high doses of *Cucumis metuliferus* fruit peel preparations. Further extended-duration toxicological evaluations (exceeding 6 months) focusing specifically on hepatic and renal mechanisms, oxidative stress markers, and post-treatment recovery phases are recommended to fully establish long-term human safety.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this study.

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