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ORIGINAL ARTICLE

# Lipid profiling and toxico-pathological assessment of the subacute oral administration of the slime extract of *Archachatina marginata* in rats

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## Abstract

**Purpose** Snail slime is essential in traditional and folkloric medicine, but empirical data on its toxicity is limited. Therefore, the present study assessed rats' toxicopathology and lipid profile after oral sub-acute exposure to *Archachatina marginata* slime extracts.

**Method** The rats were assigned into five (5) groups (n = 10) and treated for 28 days with distilled water and slime extract (125, 250, and 500 mg/kg bw). In addition, a follow-up group received the snail slime at 500 mg/kg bw and was observed post-administration for 14 days. After the treatment period, the rats were sacrificed, and the blood and vital organs were harvested for biochemical analyses which includes lipid profile, liver and kidney function, oxidative stress indices, and histopathology.

**Results** The results revealed that the slime of *A. marginata* did not remarkably affect the liver and kidney function indices and Castelli's Risk Index II (CRI-II) was < 3.0 across all groups. However, histopathology showed hepatocellular damage in the female rats, which resolved following the cessation of treatment. Furthermore, there were cellular changes, including mild inflammation and cellular degeneration of rat tissues across the treatment groups.

**Conclusion** The findings warrant further safety assessments to bolster the medicinal properties of *A. marginata* slime.

**Keywords** Hepatotoxicity · Kidney function · Medicinal biochemistry · Nephrotoxicity · Snail slime · Zootherapy

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## Introduction

Traditional remedies have been increasingly popular in recent decades. According to the World Health Organization (WHO), the majority of people use plant- and animal-based (zootherapy) medicine since it is less expensive and practical, with fewer or no side effects [1, 2]. Zootherapy uses medications manufactured or obtained from animals or their products [3]. Recently, zootherapy has become a primary focus of researchers attempting to study the efficacy of animals or their products for therapeutic purposes. Thus making the toxicological evaluation of these medicinal products imperative. For example, there is evidence to suggest the adverse effect of therapeutic animals used in traditional remedies [4].

In some parts of Africa, the African giant land snails (*Archachatina marginata*) are helpful in managing various diseases, such as allergies, asthma, anemia, and hypertension [5]. Meanwhile, snail slime is an unusual ingredient

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in cosmetics due to the presence of biochemical substances such as elastin, allantoin, hyaluronic acid, and collagen. These biochemical substances have skin care properties capable of repairing fine lines and wrinkles while lessening the harmful effects of free radicals [6]. Furthermore, several studies have examined the pharmacological qualities and bioactive properties of snail formulations for antioxidant, antibacterial, antifungal [7, 8], analgesic [9], anti-inflammatory [10], and anticancer properties [11]. Moreover, snail slime has several applications in traditional medicine systems and the cosmetic industry [12, 13], but scientific evidence of its toxicity or safety profile is limited. Therefore, the present study evaluated the toxicopathology and lipid profile in rats subacutely exposed to *Archachatina marginata* slime extract. To the best of our knowledge, this may be the first reporting the toxicopathology effects of slime extract in rats.

## Materials and methods

### Snail procurement and slime extraction

The snails (*A. marginata*) used in this study were purchased from Oje market in Ibadan, Oyo state, Nigeria. The snails weighed 250–300 g and were identified by Professor Lameed S. A. Gbolagade in the Department of Wildlife and Ecotourism Management, University of Ibadan, Oyo State, Nigeria. A plastic basket with covers was used as a house for the snails, while moist leaves and sand served as bedding. Intermittently, tap water was sprinkled on the snails to maintain hydration. They were kept for two months and fed pawpaw, bananas, watermelon, and plantain leaves [14]. The snails were thoroughly rinsed to remove dirt and contamination. They were stimulated to secrete slime by placing them in water at 32 °C. Gently stroking the snails' footplates for 1 to 5 min, they emerged from their shells and began to secrete between 1 and 5 mL of slime. An equal amount of water was added to the collected slime, which was then swirled for 1 h and centrifuged for 10 min at 3500 rpm. The supernatant was then collected and frozen [15].

### Experimental animals

Fifty Wistar rats of both sexes (4 to 6 weeks old) with weights ranging from 100 to 120 g were utilized for this study. The rats had free access to feed and clean water. The animals were housed under standard conditions in the animal house of the Department of Biochemistry, Landmark University, Omu-Aran, Kwara State. All animals were acclimatized to their new environment for 1 week before the commencement of the study.

### Subacute oral toxicity testing

Wistar rats of both sexes were assigned randomly to five groups ($n = 10$). Each group consisted of five males and five females, separately housed. The animals were grouped as control and *A. marginata* slime extract (125, 250, and 500 mg/kg bw). The fifth group was a follow-up (slime FU) given the snail slime at 500 mg/kg bw and was observed for 14 days post-treatment. The rats were orally exposed to the slime extract for 28 days, and the weight was taken weekly. On day 29, the rats were euthanized (except the follow-up group), and the blood and organs (kidney, brain, stomach, testes, ovaries, heart, spleen, and liver) were excised. Plasma was prepared for the blood sample while the organs were homogenized and centrifuged, and the supernatant was collected for biochemical analysis. A portion of the organs was preserved in 10% buffered neutral formalin (BNF) for histology examinations [16]. The follow-up group was euthanized 14 days after the cessation of treatment. The dose selection was premised on a preliminary study that determined the oral $LD_{50}$ to be > 5000 mg/kg bw in rats. The care, use, and treatment of the rats were carried out as described and approved by the Landmark University Ethical Committee. The ethical approval code is LUAC/BCH/2022/004A.

Acute oral toxicity testing of snail slime aqueous extract to determine the LD50 was carried out using the Limit test as documented by OECD 425 guidelines. This limit test was carried out by firstly dosing 5 Wistar rats with 2000 mg/kg body weight of aqueous snail slime extract. The test animals were then observed for the 1st 30 min and then for 4 h. Observations such as changes in the skin, feces, urine, sleep, respiration, tremors, and itching were recorded on a regular basis for 14 days. No mortality was recorded for the 1st dose of 2000 mg/kg body weight then the second limit test of 5000 mg/kg body weight was administered to the test animals. They were observed for the 1st 30 min and then for 4 h, and observations were recorded on a regular basis for 14 days. On the 15th day, animals were killed [17].

### Biochemical assessment

The following biochemical indices were determined by using a commercial reagent assay kits (Randox Laboratories, Crumlin, UK): aspartate aminotransferase (AST) at 340 nm, alanine amino transferase (ALT) at 340 nm, gamma-glutamyl transferase (GGT) at 405 nm, creatinine at 490 nm, urea at 546 nm, sodium (Na) at 630 nm, potassium (K) at 630 nm, total cholesterol (TC) at 505 nm, triglycerides (TAGs) at 505 nm, and high-density lipoprotein (HDL-C) at 546 nm. Other assays included total protein performed using a biuret method as described by Gornall, et al. [18]; alkaline phosphatase (ALP) was determined by the method of Wright,

et al. [19]; and glutathione-S-transferase (GST) was determined at 340 nm according to the method of Habig, et al. [20]. For the assays, absorbance was measured using a UV/Vis double-beam spectrophotometer (VWR International, Pennsylvania, USA).

### Histopathological examination

Trimmed parts of the rat organs (kidneys, brain, liver, stomach, spleen, ovaries/testis, and heart) were fixed in 10% BNF for histological examination. The formalin-fixed organs were processed and embedded in paraffin wax, and then stained with haematoxylin and eosin for microscopic assessment using a light microscope (Olympus CX21) with × 10 and 40 objectives. Photomicrographs were taken using a camera (AM-Scope 500) mounted light microscope [21].

### Data analysis

Data analysis was performed on a GraphPad Prism 9.0 software package (San Diego, California USA) and the results are presented as mean ± standard deviation (SD). Experimental groups were compared using a two-way analysis of variance (ANOVA). Statistical significance was defined as $p < 0.05$.

## Results

### Body weight and relative organ weight

There was a notable increase in the body weights of the group that received slime 500 mg/kg bw and the male follow-up group (Fig. 1). In the male groups, the 500 mg/kg bw treatment had a remarkable ($p < 0.05$) increase in liver-to-body weight ratio in comparison with the control (Table 1).

### Effects of *Archachatina marginata* slime extract on the level of total protein

In the female follow-up group (500 mg/kg bw), the plasma and liver total protein remarkably ($p < 0.05$) increased when compared with the control (Fig. 2A, C). Also, the male rat kidney (125 mg/kg bw) had a remarkable ($p < 0.05$) increase in total protein in comparison with the male follow-up group (500 mg/kg bw) (Fig. 2B).

### The effects of *A. marginata* slime extract administration on rat liver

The ALT level was elevated ($p < 0.05$) in the male treatment groups (250 mg/kg bw and 500 mg/kg bw) as well as in the female (500 mg/kg bw) and follow-up groups compared with the other groups (Fig. 3A). AST levels in both the males and females did not show any remarkable ($p > 0.05$) differences in all treatment groups (Fig. 3B). The GGT level in the female high-dose (500 mg/kg bw) and follow-up groups was remarkably ($p < 0.05$) increased in comparison with the other treatment groups (Fig. 3D). Meanwhile, there was a remarkable ($p < 0.05$) increase in ALP levels in the male high-dose (500 mg/kg bw) group compared with the other treatment groups (Fig. 3C). The levels of GST in the male low-dose (125 mg/kg bw) group were remarkably ($p < 0.05$) increased, but lowered ($p < 0.05$) in the male high-dose (500 mg/kg bw) group in comparison with the other treatment groups (Fig. 3E). The AST/ALT ratio of the male low-dose (125 mg/kg bw) and the female (125, 250, and 500 mg/kg bw) treatment groups was > 1.5 (Table 2).

### The effects of *Archachatina marginata* slime extract administration on rat kidney

There was no remarkable ($p > 0.05$) difference in creatinine, urea, and potassium levels across the male and

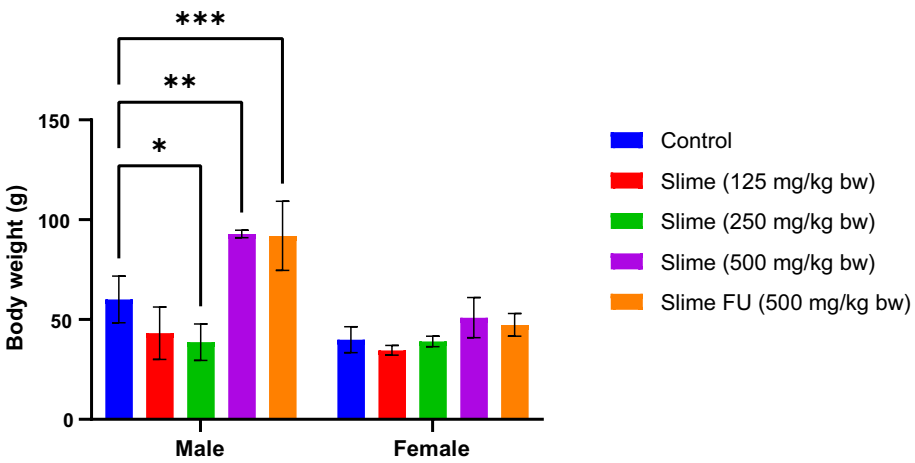


**Fig. 1** Effects of *Archachatina marginata* slime extract on the body weight of Wistar rats. Each value represents a mean of five replicates ± SD. Data with asterisks are significantly different at $p < 0.05$. Data with asterisks are significantly different at $p < 0.05$. * is significant at $p < 0.05$, **, ***, and **** at $p < 0.01$, $p < 0.001$, and $p < 0.0001$ versus control, respectively

**Table 1** Effect of *Archachatina marginata* slime extract on organ to body weight ratio in male and female rats

| | | Control | Slime (125 mg/kg bw) | Slime (250 mg/kg bw) | Slime (500 mg/kg bw) | Slime FU (500 mg/kg bw) |
|---|---|---|---|---|---|---|
| Liver | M | $3.40 \pm 0.16^{a}$ | $3.16 \pm 0.15^{a}$ | $3.44 \pm 0.12^{a}$ | $4.43 \pm 0.08^{b}$ | $3.58 \pm 0.16^{a}$ |
| | F | $4.11 \pm 0.47^{a}$ | $3.70 \pm 0.23^{a}$ | $3.35 \pm 0.12^{a}$ | $3.58 \pm 0.09^{a}$ | $3.30 \pm 0.05^{a}$ |
| Kidney | M | $0.63 \pm 0.04^{a}$ | $0.65 \pm 0.03^{a}$ | $0.67 \pm 0.03^{a}$ | $0.64 \pm 0.04^{a}$ | $0.59 \pm 0.04^{a}$ |
| | F | $0.64 \pm 0.06^{a}$ | $0.89 \pm 0.24^{a}$ | $0.68 \pm 0.04^{a}$ | $0.64 \pm 0.03^{a}$ | $0.64 \pm 0.01^{a}$ |
| Brain | M | $0.65 \pm 0.03^{a}$ | $0.69 \pm 0.05^{a}$ | $0.80 \pm 0.05^{a}$ | $0.65 \pm 0.03^{a}$ | $0.72 \pm 0.05^{a}$ |
| | F | $0.93 \pm 0.24^{a}$ | $1.00 \pm 0.26^{a}$ | $0.77 \pm 0.02^{a}$ | $0.78 \pm 0.03^{a}$ | $0.76 \pm 0.03^{a}$ |
| Heart | M | $0.35 \pm 0.01^{a}$ | $0.36 \pm 0.03^{a}$ | $0.39 \pm 0.01^{a}$ | $0.35 \pm 0.02^{a}$ | $0.40 \pm 0.03^{a}$ |
| | F | $0.35 \pm 0.04^{a}$ | $0.59 \pm 0.20^{a}$ | $0.35 \pm 0.02^{a}$ | $0.35 \pm 0.02^{a}$ | $0.37 \pm 0.01^{a}$ |
| Testes/ovaries | M | $1.30 \pm 0.06^{a}$ | $1.32 \pm 0.09^{a}$ | $1.06 \pm 0.21^{a}$ | $1.23 \pm 0.03^{a}$ | $1.36 \pm 0.11^{a}$ |
| | F | $0.07 \pm 0.01^{a}$ | $0.10 \pm 0.02^{a}$ | $0.08 \pm 0.01^{a}$ | $0.08 \pm 0.01^{a}$ | $0.07 \pm 0.01^{a}$ |
| Spleen | M | $0.31 \pm 0.01^{a}$ | $0.30 \pm 0.03^{a}$ | $0.43 \pm 0.05^{a}$ | $0.33 \pm 0.04^{a}$ | $0.37 \pm 0.03^{a}$ |
| | F | $0.39 \pm 0.03^{a}$ | $0.69 \pm 0.24^{a}$ | $0.39 \pm 0.03^{a}$ | $0.43 \pm 0.03^{a}$ | $0.39 \pm 0.03^{a}$ |

Each value represents a mean of five replicates ± SD. Values with different superscripts are significantly different at $p < 0.05$ across the row. a is significant at $p < 0.05$, b at $p < 0.01$, c at $p < 0.001$, and d at $p$ versus 0.0001 versus control

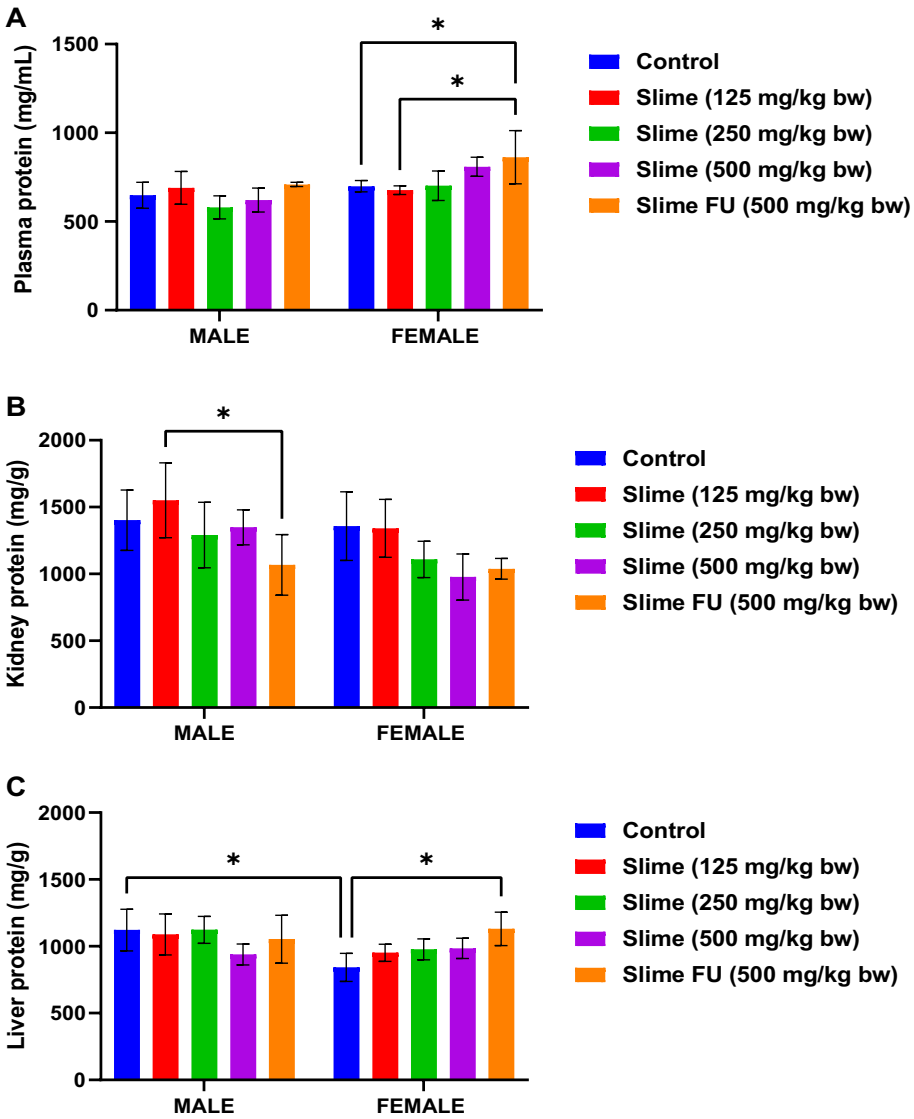


**Fig. 2** Effects of *Archachatina marginata* slime extract administration on rat plasma, kidney and liver total protein levels. **A** Plasma protein, **B** kidney protein, and **C** liver protein. Each value represents a mean of five replicates ± SD. Data with asterisks are significantly different at $p < 0.05$. * is significant at $p < 0.05$, **, ***, and **** at $p < 0.01$, $p < 0.001$, and $p < 0.0001$ versus control, respectively

female treatment groups (Fig. 4A, B). However, the follow-up (500 mg/kg bw) group of the male had a remarkably ($p < 0.05$) reduced urea level compared with the male mid-dose (250 mg/kg bw) (Fig. 4C). Furthermore, the follow-up (500 mg/kg bw) groups had significantly reduced sodium levels compared with other treatment groups (Fig. 4D).

## The effects of *Archachatina marginata* slime extract administration on rat lipid profile

The lipid profile in the male follow-up (500 mg/kg bw) group was remarkably ($p < 0.05$) increased in comparison with the male treatment groups and control group (Fig. 5). The ratio of LDL-C/HDL-C (Castelli's index II) was estimated at 3.0 in both the male and female treatment groups (Table 3).

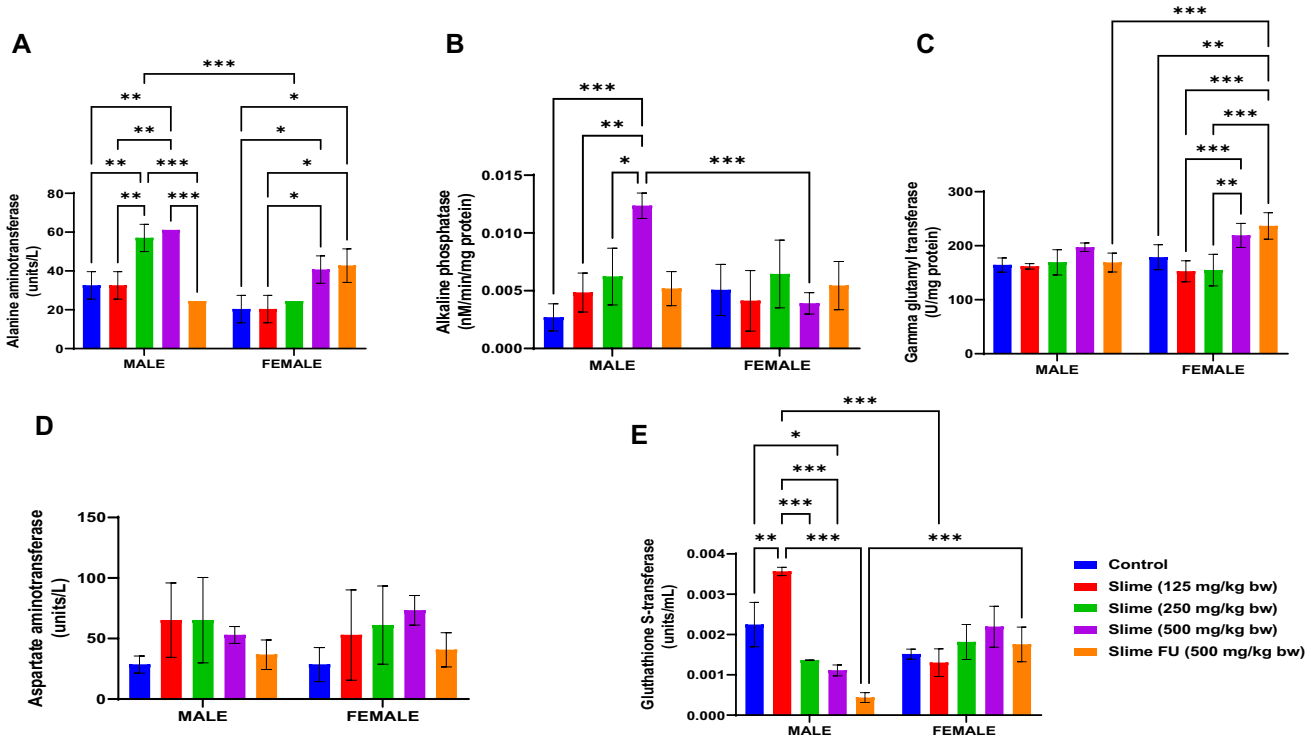


**Fig. 3** Effects of *Archachatina marginata* slime extract administration on liver function parameters of Wistar rats. **A** Alanine aminotransferase, **B** aspartate aminotransferase, **C** alkaline phosphatase, **D** gamma-glutamyl transferase, and **E** gluthathione S-transferase. Each value represents a mean of five replicates ± SD. Data with asterisks are significantly different at $p < 0.05$. * is significant at $p < 0.05$, **, ***, and **** at $p < 0.01$, $p < 0.001$, and $p < 0.0001$ versus control, respectively

**Table 2** AST/ALT ratio of Wistar rats treated with *Archachatina marginata* slime extract

| | Control | Slime (125 mg/kg bw) | Slime (250 mg/kg bw) | Slime (500 mg/kg bw) | Slime FU (500 mg/kg bw) |
|---|---|---|---|---|---|
| Male | $0.59 \pm 0.08^a$ | $1.61 \pm 0.63^a$ | $0.98 \pm 0.22^a$ | $1.28 \pm 0.37^a$ | $1.17 \pm 0.44^a$ |
| Female | $0.87 \pm 0.32^a$ | $1.58 \pm 0.58^a$ | $1.58 \pm 0.42^a$ | $1.83 \pm 0.25^a$ | $0.62 \pm 0.09^a$ |

Each value represents a mean of five replicates ± SD. Values with different superscripts are significantly different at $p < 0.05$ across the row

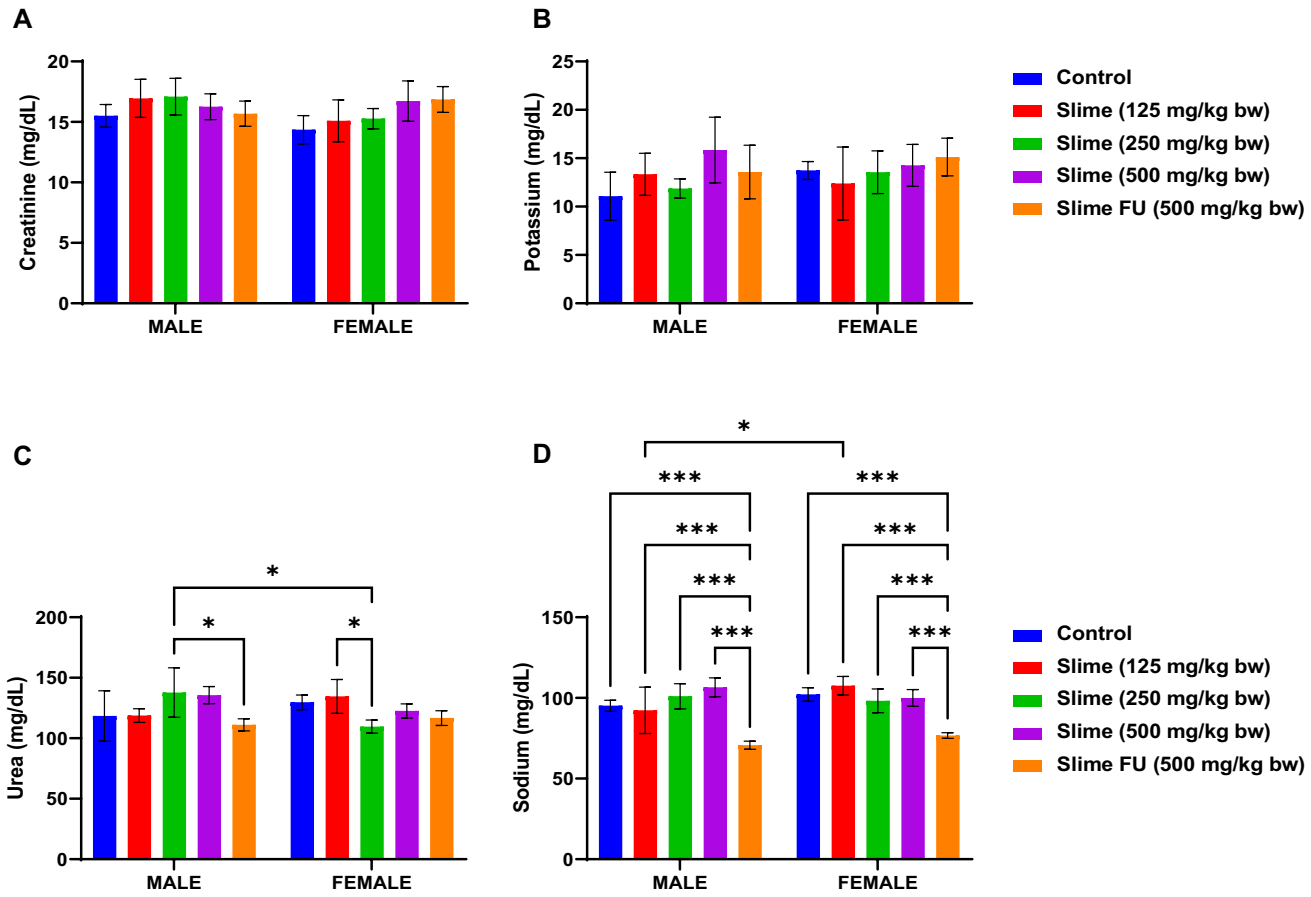


**Fig. 4** Effects of *Archachatina marginata* slime extract administration on kidney function parameters of Wistar rats. **A** Plasma creatinine, **B** plasma potassium, **C** plasma urea, and **D** plasma sodium. Each value represents a mean of five replicates ± SD. Data with asterisks are significantly different at $p < 0.05$. * is significant at $p < 0.05$, **, ***, and **** at $p < 0.01$, $p < 0.001$, and $p < 0.0001$ versus control, respectively

## Effects of oral subacute exposure to *A. marginata* slime extract on rat organ morphology

A pathologist blinded to the treatments carried out the gross pathological examination of the rat organs. The organs examined included the kidney, liver, brain, stomach, heart, spleen, and ovaries (Figs. 6, 7, 8, 9, 10, 11, 12). The histopathology details are as follows: In the kidney, the control and male slime FU groups show no observable lesion or cellular changes, the glomeruli appeared normal with adequate capsular spaces. Female and male treatment (125, 250, and 500 mg/kg bw) groups presented with mild, severe to moderate degenerative changes (*c*) and necrotic areas (*n*), respectively. There were evidence of hemorrhage characterized with inflammatory cells. Female slime FU showed no significant cellular damages (Fig. 6).

In the liver, the male and female control, treatment (125 and 500 mg/kg bw), and slime FU groups showed no significant observable lesions or cellular changes. The male middose (250 mg/kg bw) group showed hepatocellular necrosis and cellular inflammation. Female treatment (125, 250, and 500 mg/kg bw) groups showed evidence of mild necrotic changes (*n*) and hepatocyte degeneration with moderate inflammatory reactions. In addition, moderate cellular distortion was observed in female treatment (125 and 250 mg/kg bw) groups (Fig. 7). In the stomach, the micrographs showed well-outlined arrays of normal gastrointestinal tissues with no significant observable cyto-architectural distortion in the female and male groups (Fig. 8).

In the testes, the control, treatment (125 and 500 mg/kg bw) and slime FU groups showed a normal cellular pattern of the testis, but the treatment (250 mg/kg bw) group

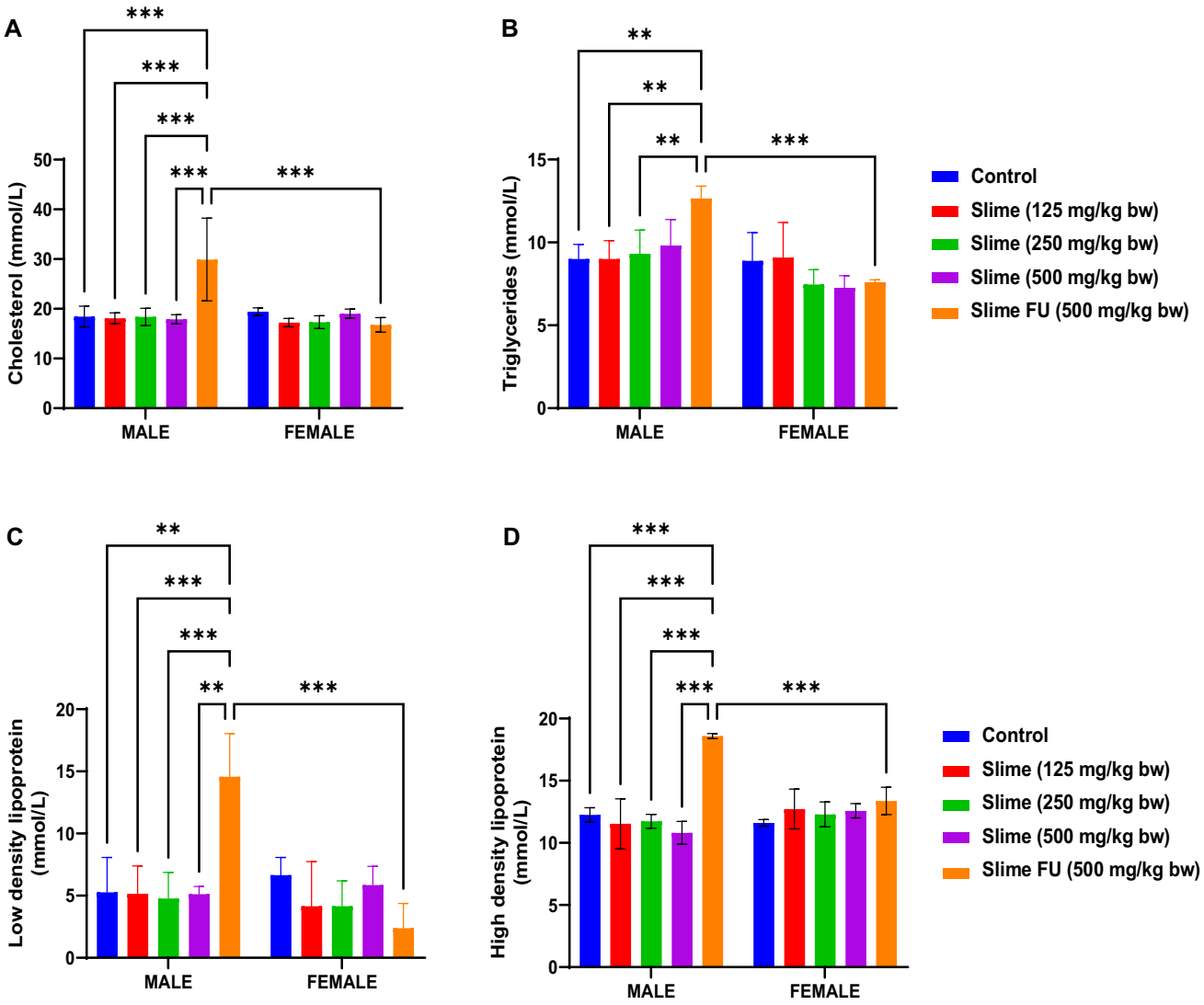


**Fig. 5** Effects of *Archachatina marginata* slime extract administration on lipid profile parameters in Wistar rats. **A** Cholesterol, **B** triglycerides, **C** low-density lipoprotein, and **D** high-density lipoprotein). Each value represents a mean of five replicates ± SD. Data with asterisks are significantly different at $p < 0.05$. * is significant at $p < 0.05$, **, ***, and **** at $p < 0.01$, $p < 0.001$, and $p < 0.0001$ versus control, respectively

showed marked cellular degenerative changes characterized by reduced spermatogenetic activity and necrotized testis with notable loss of interstitial cells (Fig. 9). In the ovaries, the control, treatment (500 mg/kg bw) and slime FU groups showed dilated blood vessels, inter-stroma adipose connective tissue, normal oviduct and primordial follicle at various stages of development. Treatment (125 and 250 mg/kg bw) groups showed atrophied ovarian cells (xx), some wasted follicles, and mild degenerative changes as well as fibrosis (Fig. 9).

In the brain, the male and female control, treatment (125 and 500 mg/kg bw), and slime FU groups showed no significant degenerative changes. Meanwhile, the male treatment (250 mg/kg bw) group showed moderate infiltrations of inflammatory cells (T), with cellular degenerations (Fig. 10).

In the heart, the male and female control, treatment (500 mg/kg bw), and slime FU groups showed no observable cellular damage, degenerative changes, or necrosis. The male and female treatment (125 and 250 mg/kg bw) groups showed mild to severe wavy cardiac muscle (**wv**) cells with apparent

**Table 3** Ratio LDL-C/HDL-C (Castelli's index II) in rats treated with *Archachatina marginata* slime extract

| | Control | Slime (125 mg/kg bw) | Slime (250 mg/kg bw) | Slime (500 mg/kg bw) | Slime FU (500 mg/kg bw) |
|---|---|---|---|---|---|
| Male | $0.43 \pm 0.10^{a}$ | $0.48 \pm 0.10^{a}$ | $0.41 \pm 0.09^{a}$ | $0.48 \pm 0.04^{a}$ | $0.96 \pm 0.16^{b}$ |
| Female | $0.57 \pm 0.06^{a}$ | $0.35 \pm 0.16^{a}$ | $0.35 \pm 0.09^{a}$ | $0.46 \pm 0.06^{a}$ | $0.19 \pm 0.09^{a}$ |

Each value represents a mean of five replicates ± SD. Data with superscript b are significantly different at $p < 0.05$

necrotic (x) cells, and the treatment group (250 mg/kg bw) showed severe myofibril degeneration with decreased striation and fading nuclei (Fig. 11).

In the spleen, the male and female control groups showed well-outlined arrays of normal tissue without any observable cyto-architectural distortion. However, the male and female treatment (500 mg/kg bw) and slime FU groups showed mild degenerative changes. The male and the female treatment (125 and 250 mg/kg bw) groups showed sinusoid hemorrhage, severe necrotized and de-organized tissues (Fig. 12).

## Discussion

Snails are essential components in traditional and new-generation medicines, thus confirming their ethnopharmacological and therapeutic potential [12, 13]. Studies have shown that snail slime has some significant pharmacological activities, making it attractive for inclusion in some drugs and skin care products [6, 9, 10]. While various studies report snail slime's pharmacological properties and chemical composition, its toxicity profiling is limited. Subacute toxicity studies estimate the adverse effects of a substance after repeated administration for 28 days, and they provide helpful information about a substance's cumulative toxicity on physiology and target organs. In an acute toxicity study, substances with no toxic effects may become lethal with prolonged or repeated exposure, even at low doses [22]. Hence, we evaluated the toxicopathology and lipid profile of rats following a subacute oral exposure to the slime extract of *Archachatina marginata* using the OECD 407 guidelines [16].

Body and organ weights are important toxicological parameters used to detect target organs of test substances and the pathophysiological state of the animals. Various biological factors can cause a significant increase or decrease in body weight, including alteration of growth hormones and neurotransmitters responsible for food consumption, reduced feed palatability, and environmental factors [23, 24]. The remarkable decrease in the male mid-dose (250 mg/kg bw) and remarkable increase in the male high-dose and slime FU (500 mg/kg bw) groups compared with the male control group might be due to one of the factors mentioned above. There was also a remarkable increase in the liver weight of the male high-dose (500 mg/kg bw) group compared with the control and the slime FU (500 mg/kg bw) groups. This indicates that the oral administration of *A. marginata* slime extract at 500 mg/kg bw might have adversely affected the liver of the males at

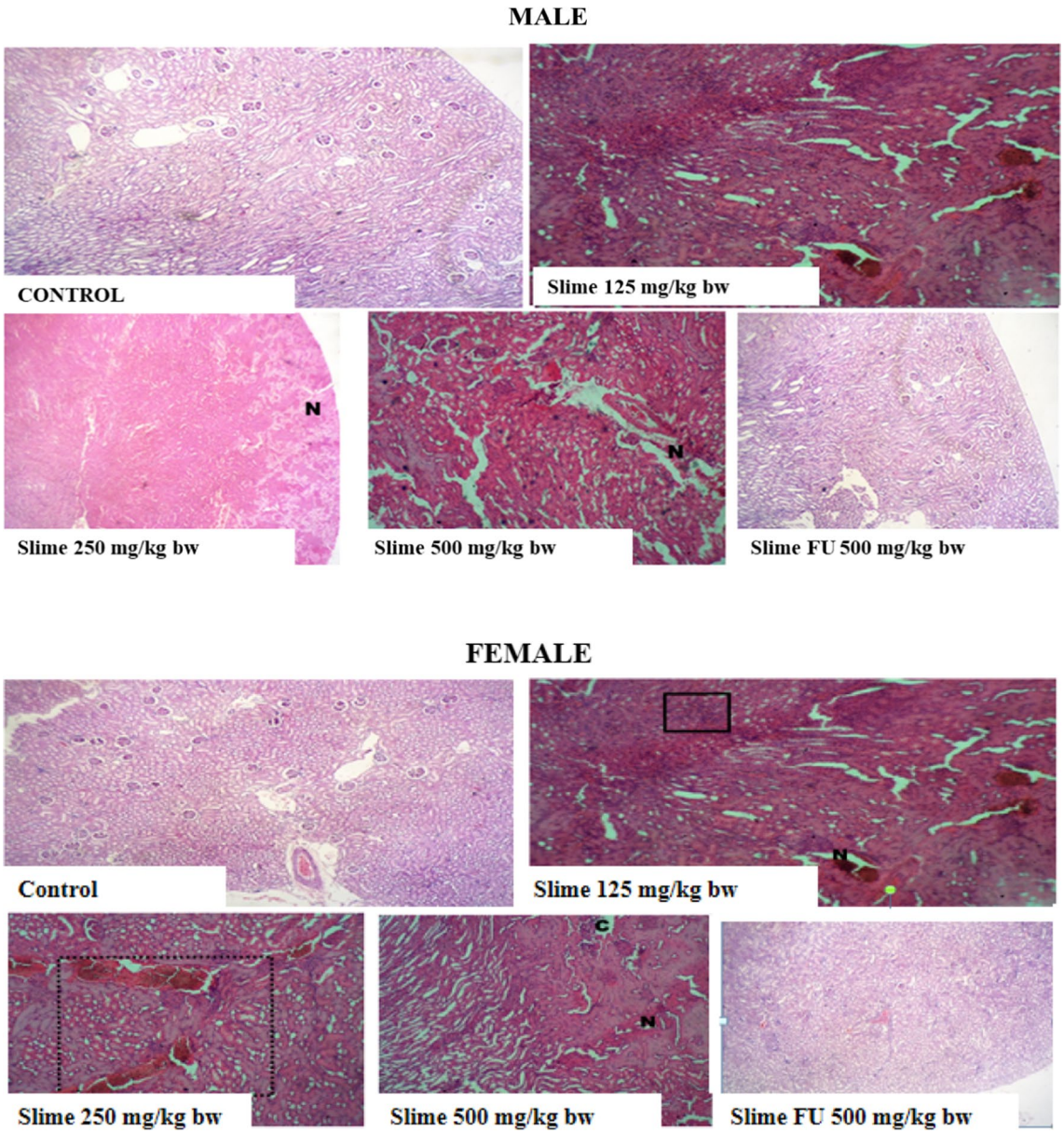


**Fig. 6** Representative photomicrographs of male and female rat kidney following a subacute oral toxicity study of *Archachatina marginata* slime extract. (H & E, × 10). (*N* = necrotic areas, *C* = degenerative changes)

the time of administration when compared with the liver of the male follow-up group at the exact dosage.

The liver is the leading site for protein synthesis; the total protein concentration may give an idea of the functional status of hepatocytes [25]. Elevated levels or decreased total protein levels may indicate damage to the liver or kidney tissues. Changes in plasma protein levels can be due to alterations in plasma water concentration or alterations in one of the plasma protein concentrations [26]. Furthermore, oral administration of *A. marginata* slime extract increased the levels of total protein in the plasma and liver of the female follow-up group (500 mg/kg bw) as well as in the kidney of the male low-dose group (125 mg/kg bw). This may indicate an alteration in the functioning capacity of the liver or kidney in the female follow-up and male low-dose groups.

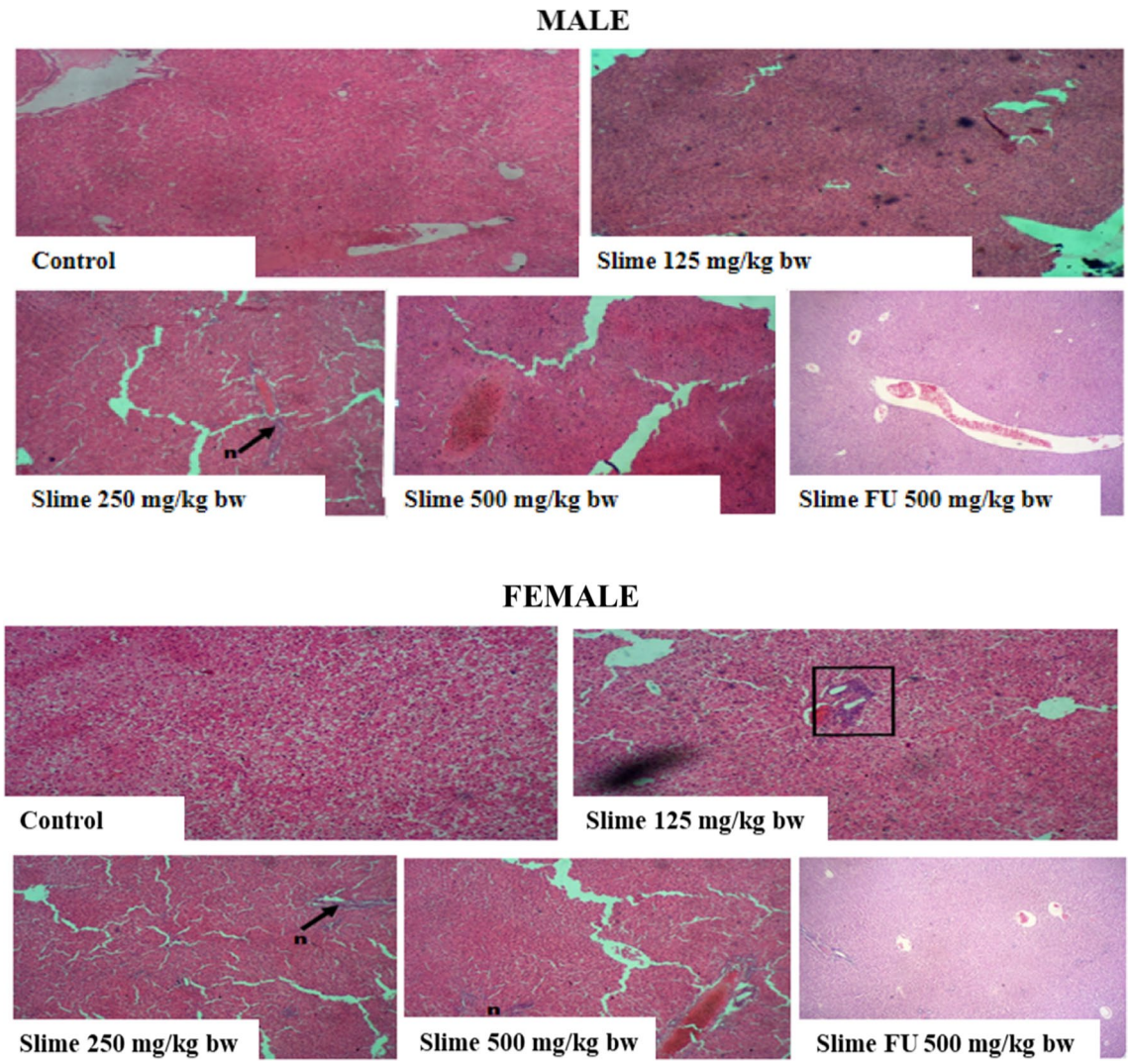


**Fig. 7** Representative photomicrographs of male and female rat liver following a subacute oral toxicity study of *Archachatina marginata* slime extract. (*H* & *E*, × 10). (*N* = necrotic areas)

In addition, the present study assessed the markers of hepatocellular injury, such as the amino transaminases (AST and ALT), ALP, GGT, and GST, to detect adverse effects of *A. marginata* slime on the liver. ALT is a specific marker enzyme of liver damage, and the mitochondrial isozyme of AST is produced in the hepatocytes. When there is liver damage (hepatitis puncture), ALT and AST leak out, causing an increased level of these enzymes in the blood. ALP increases when there is cholestasis or accumulation of bile salts, which may indicate kidney damage. An increase in ALP in conjunction with an increase in GGT shows a risk of biliary tract obstruction [27]. The AST/AST ratio may help to distinguish sites of cholestasis, also known as biliary obstruction, which is of two types: intrahepatic and extrahepatic obstruction. The female treatment (125, 250, and 500 mg/kg bw) groups had an AST/ALT ratio of > 1.5, indicating the presence of intrahepatic cholestasis. The result of the liver histopathology in the female treatment groups, which showed cellular distortions, reinforces the biochemical alteration in the ALT/AST ratio. The male low-dose (125 mg/kg bw) group also had an AST/ALT ratio of > 1.5, which correlates with its increased plasma GST level, indicating liver

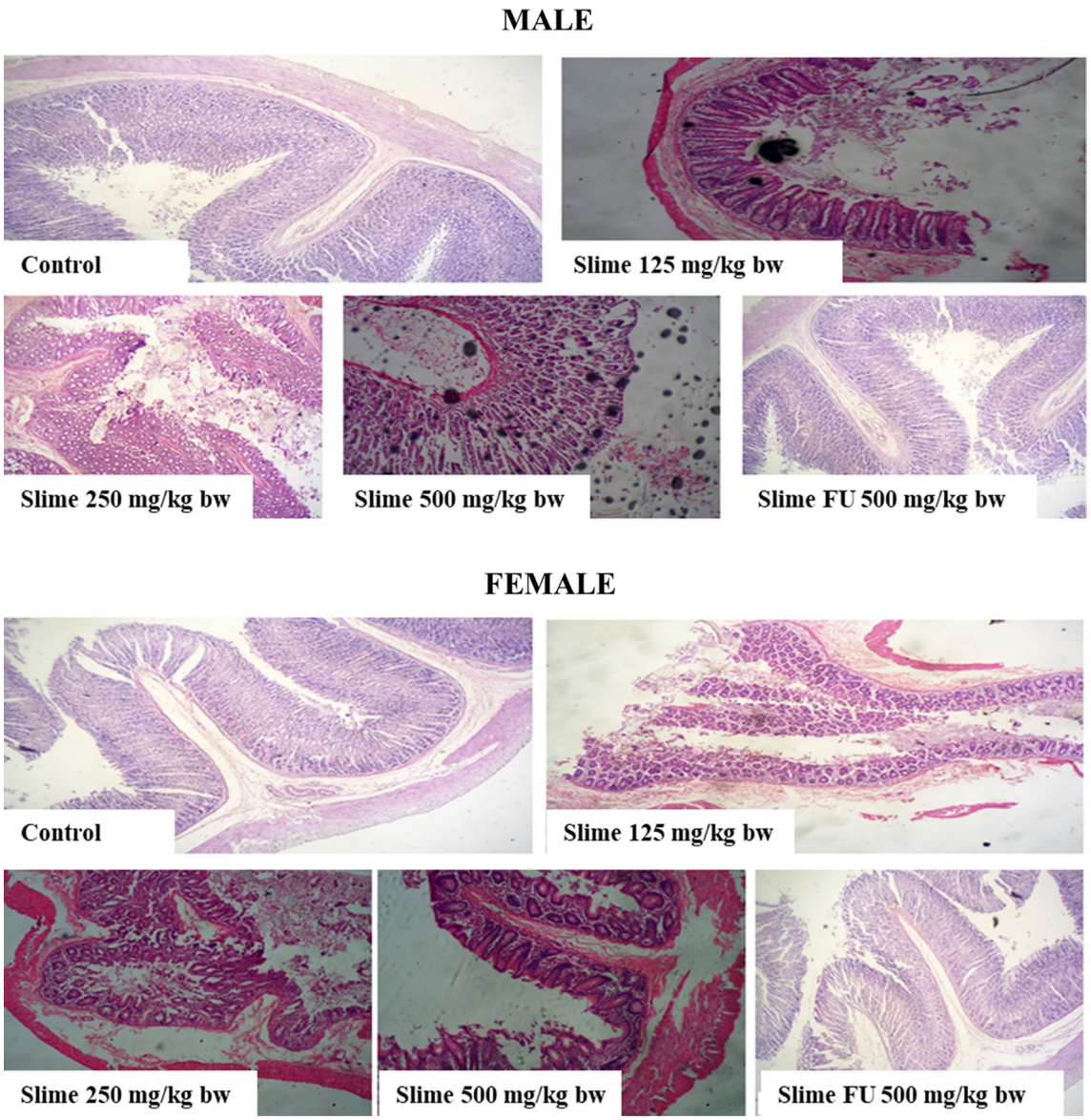


**Fig. 8** Representative photomicrographs of male and female rat stomach following a subacute oral toxicity study of *Archachatina marginata* slime extract. (*H & E*, × 10)

damage (cholestasis). Together, the data may indicate that the slime extract of *A. marginata* might have caused more toxicity to the female rat liver.

Renal damage leads to alterations in the levels of significant markers such as blood creatinine, urea, and electrolytes (sodium and potassium). Creatinine is an endogenous marker of glomerular function. It is a by-product of muscle creatinine phosphate. The liver produces urea as a part of the urea cycle and protein metabolism [28]. The kidney is the organ responsible for the clearance of creatinine and urea from the body system [29].

Meanwhile, electrolytes help maintain normal biochemical reactions and homeostatic functioning, including maintaining cell membrane functions, nerve conductivity, hormonal activity, and fluid acid–base balance. Furthermore, the kidneys play a crucial role in electrolyte and fluid homeostasis. Sodium ($Na^+$) is the most abundant extracellular electrolyte in the body, and it helps with fluid balance, osmotic regulation, and membrane potential maintenance.

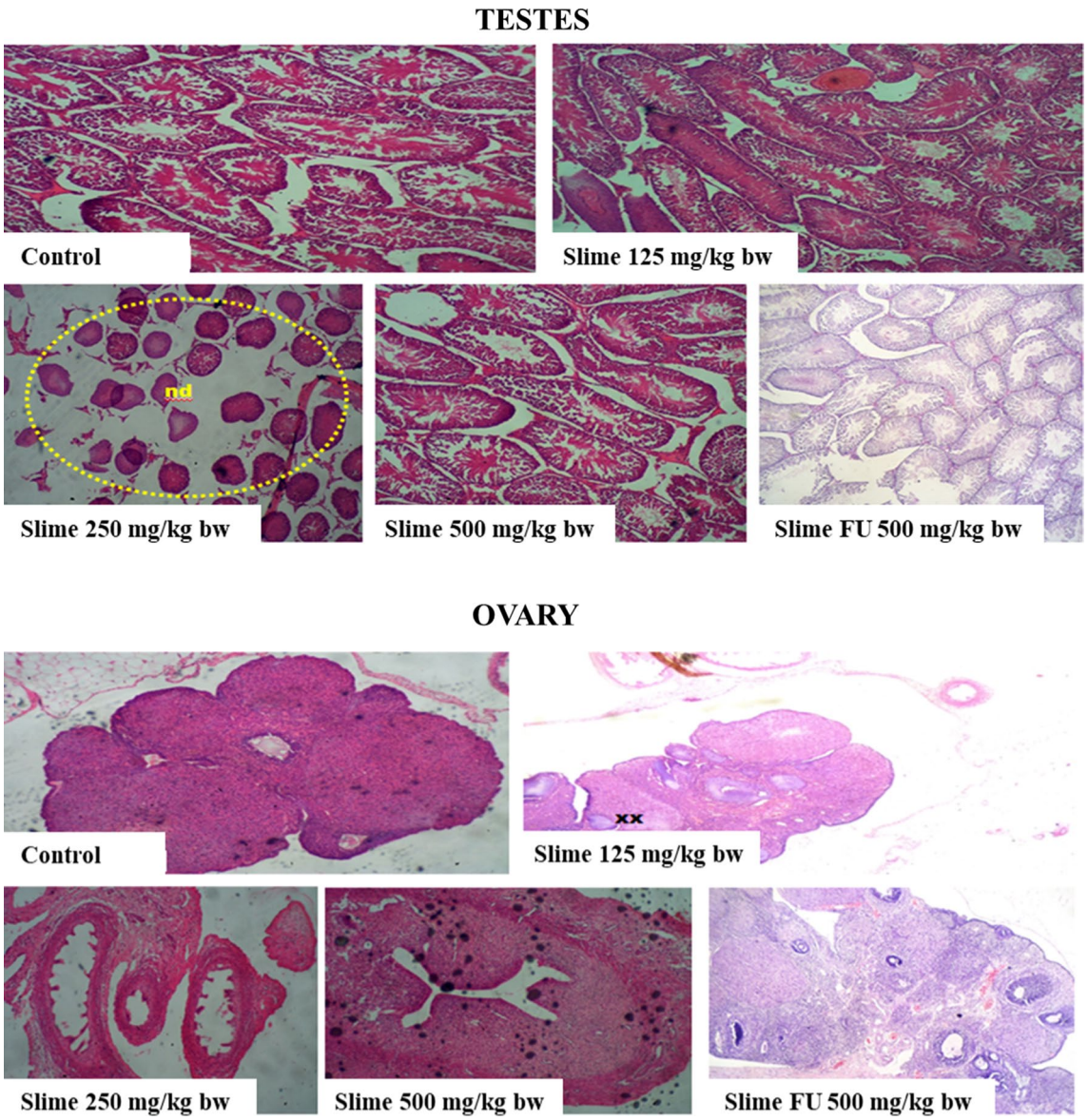


**Fig. 9** Representative photomicrographs of rat testes and ovary following a subacute oral toxicity study of *Archachatina marginata* slime extract. (*H* & *E*, × 10). (ND = cellular degenerative changes and necrotized testis, XX = atrophied ovarian cells)

Potassium ($K^+$) is the primary intracellular cation in the body, and it helps in cellular metabolism, membrane potential maintenance, neuromuscular and cardiac function [30]. The findings in this study may indicate normal renal clearance as the levels of plasma urea, and creatinine remained unchanged among the treatment and control groups for both the males and females. In addition, there was no remarkable difference in the potassium level of the male and female experimental groups compared to the control. However, the sodium level was significantly decreased in the male and female follow-up (500 mg/kg bw) groups compared with the other treatment groups. This finding may indicate alterations in fluid homeostasis and osmotic regulation consequent upon slime extract administration. Taken the results together, *A. marginata* slime extract might not have caused any severe toxicity to the rat kidneys, though the histology revealed a mild nephrotic distortion following a 28-day oral exposure to the slime extract.

Plasma lipid profiles are helpful in the assessment of the risk of cardiovascular diseases. Cholesterol, a precursor for

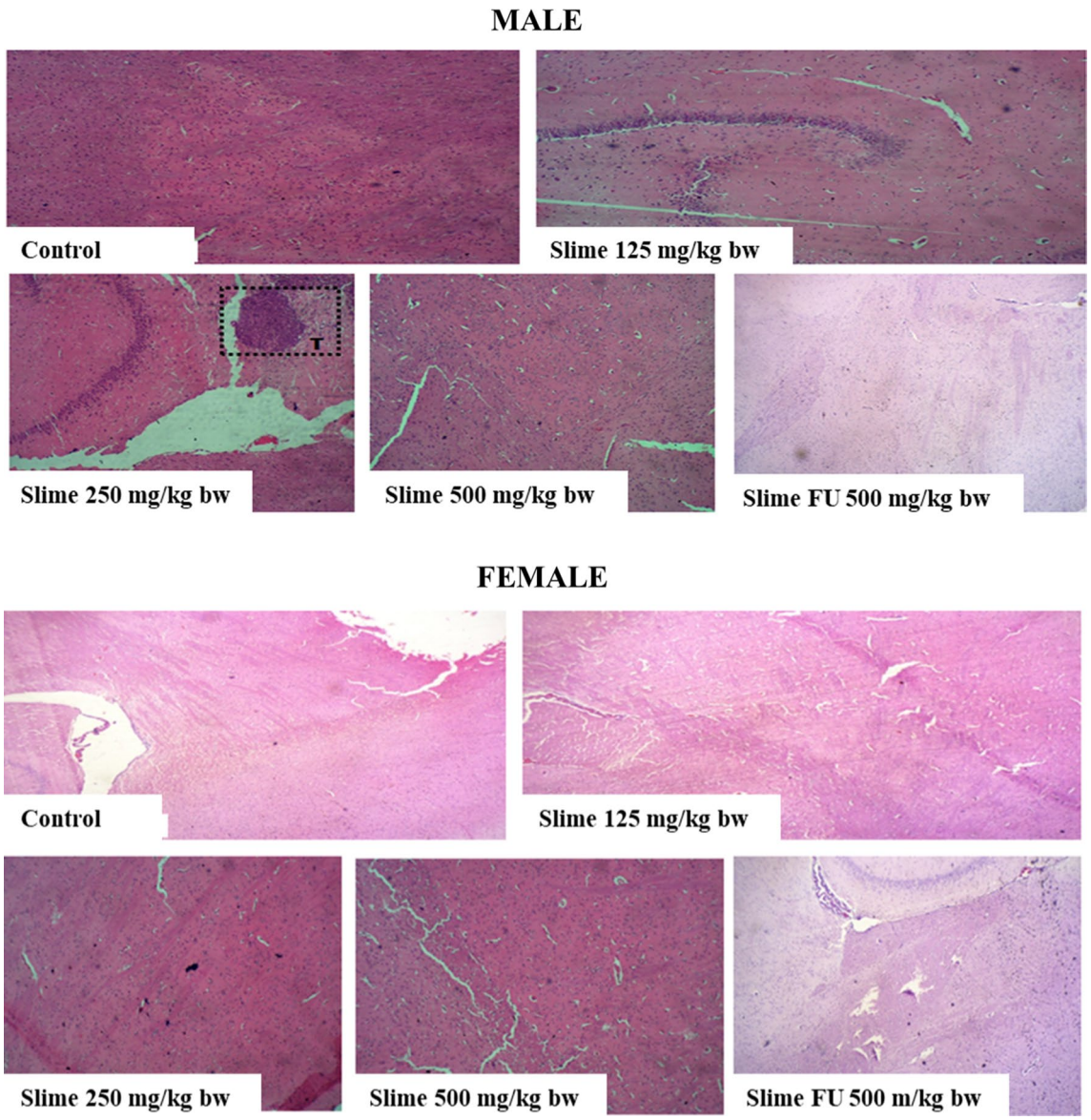


**Fig. 10** Representative photomicrographs of male and female rat brain following a subacute oral toxicity study of *Archachatina marginata* slime extract. (*H* & *E*, × 10). (T = infiltrations of inflammatory cells)

hormones, bile acid production, and vitamins, is essential to most biological membranes [31]. The oral administration of *A. marginata* slime extract decreased total cholesterol and LDL-C across the treatment groups of males and females. However, the same parameters were remarkably increased in the male follow-up group. TAG levels slightly increased across the male treatment and follow-up groups, but the same parameter was reduced in the female groups. However, HDL-C levels in the male and female groups increased, indicating a potentially lower risk for cardiovascular diseases. The findings correlate with the atherogenic index (AI). The LDL-C/HDL-C ratio was less than 3.0 across all treatment groups in both males and females. The atherogenic index (AI) is a significant biomarker of dyslipidemia. An LDL-C/HDL-C ratio greater than 3 indicates an increased risk of atherosclerosis. The findings may relate to the shallow crude fat content of *A. marginata* mucin [32].

The histopathological examination of the rat organs showed no lesions, inflammation, or degenerative changes in the control for both males and females. However, a combination of

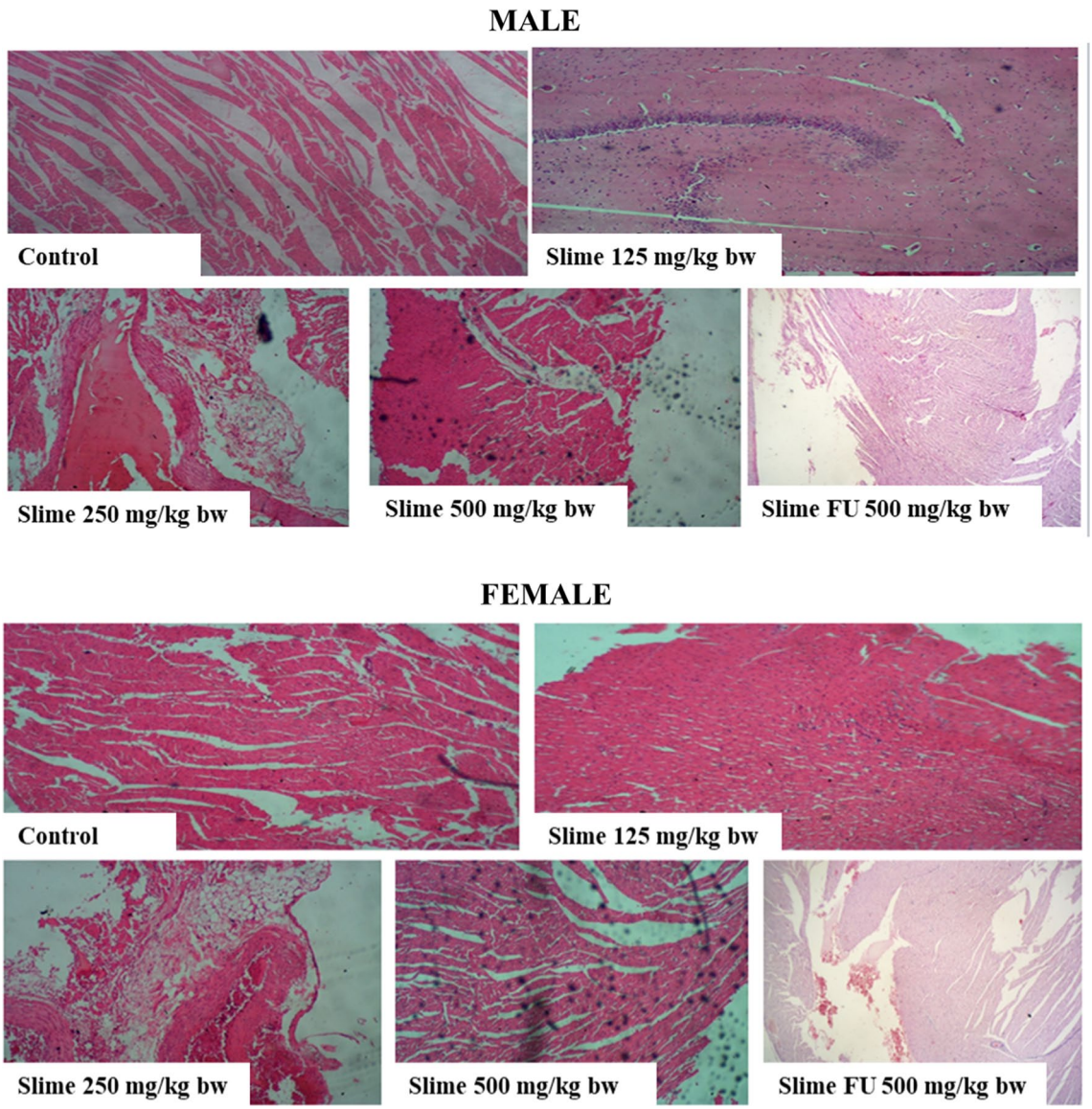


**Fig. 11** Representative photomicrographs of male and female rat heart following a subacute oral toxicity study of *Archachatina marginata* slime extract. (*H* & *E*, × 10)

mild degenerative changes such as necrosis and inflammation characterized the liver, kidney, spleen, brain, heart, ovaries, and testes following a 28-day oral administration of the slime extract of *A. marginata.* In addition, the kidney in the female follow-up group and the spleen of both the males and females showed distortions of the cellular architecture. Wavy cardiac muscles observed in the histology examinations of the heart may underscore the cardio-toxic potential of the slime extract of *A. marginata.* However, future studies would clarify the cardio-protective properties of snail slime. Meanwhile, the stomachs of both the males and females had no detectable lesions in the treatment and follow-up groups, and this finding is consistent with the gastro-protective properties of *A. marginata* slime, as earlier reported [33].

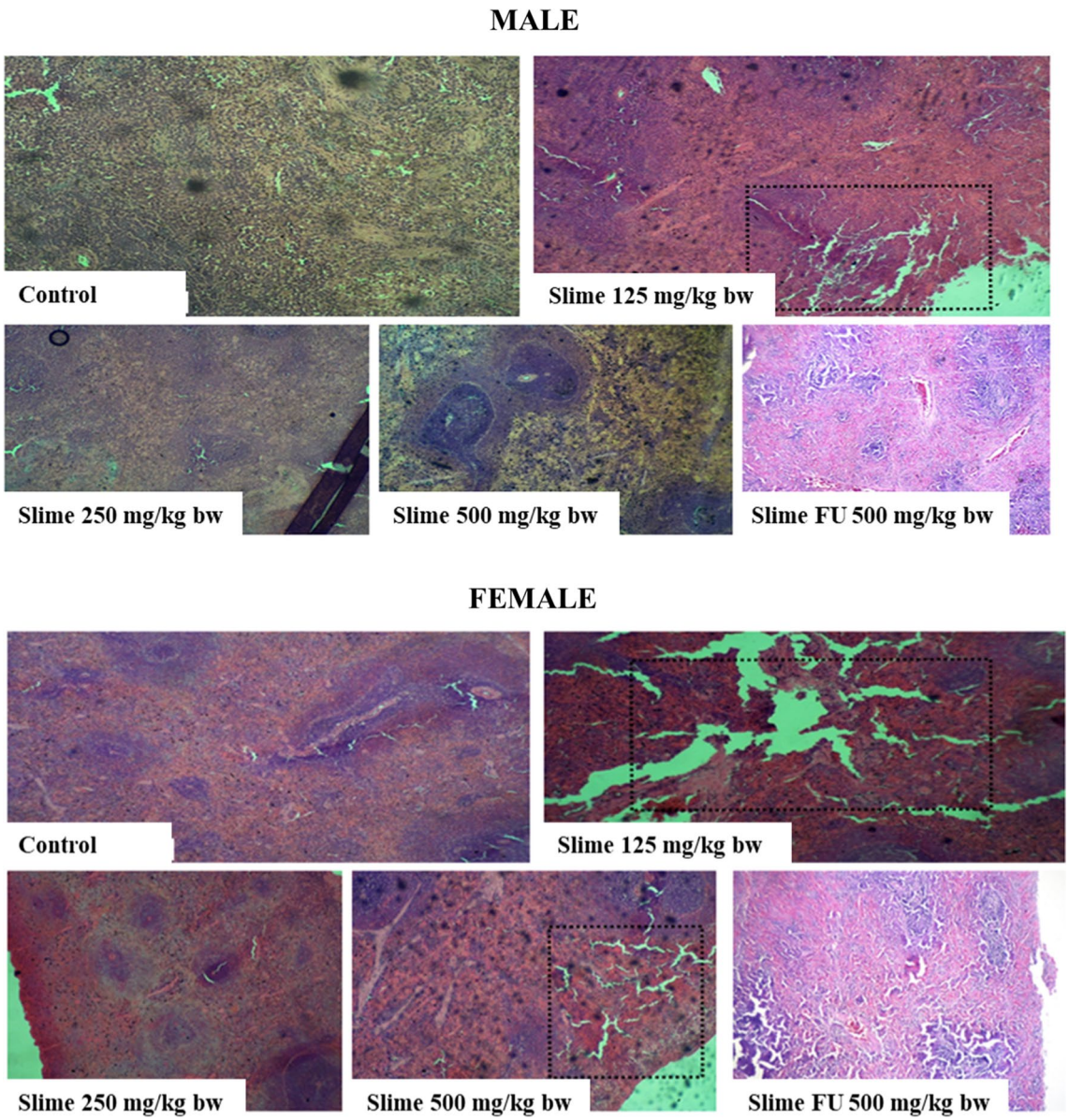


**Fig. 12** Representative photomicrographs of male and female rat spleen following a subacute oral toxicity study of *Archachatina marginata* slime extract. (*H* & *E*, × 10)

## Conclusion

A 28-day oral exposure of rats to the slime extract of *A. marginata* revealed no appreciable alterations to the lipid profile or nephrotic indices. The female rat liver showed altered biochemical indices reminiscent of hepatotoxicity but improved 14 days after treatment cessation. The risk of developing cardiovascular disorders was considerably lower than that reflected by the atherogenic index (LDL-C/HDL-C ratio), which was < 3.0. However, the slime extract of *A. marginata* caused distortions in the cellular architecture of rat organs in ways that suggest early cellular injury. The findings warrant further investigation to explore the toxicity of the slime extract of *A. marginata* using a molecular approach. Furthermore, considering the rat cellular distortions caused by a 28-day oral exposure to the snail slime extract, its consumption should be with caution.

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**Author contributions** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, OSA,

MEB and OJA; methodology, MEB, OSA; software, DER and TCB; validation, OSA and FAO; formal analysis, MEB, OSA and OJA; investigation, MEB; resources, MEB, OJA, OSA and FAO; data curation, DER, TCB and MEB; writing—original draft preparation, MEB, DER, OSA; writing—review and editing, OJA; visualization, OSA; supervision, OSA and OJA; project administration, OSA; funding acquisition, MEB. All authors have read and agreed to the published version of the manuscript."

**Availability of data and materials** The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** Morayo Elizabeth Barnabas, Damilare Emmanuel Rotimi, Tobiloba Christiana Elebiyo, Funmilayo Abimbola Okeniyi, Oluwakemi Josephine Awakan, and Oluyomi Stephen Adeyemi declare that we have no conflict of interest.

**Ethics approval and consent to participate** The care, use, and treatment of the rats were carried out as described and approved by the Landmark University Ethical Committee. The ethical approval code is LUAC/BCH/2022/004A.

**Consent for publication** Not applicable.

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