

Original Research Article

Toxicological profile of antimicrobial metabolites of endophytic fungal isolates of *Synclisia scabrida* Miers

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Abstract

Purpose: To evaluate the acute and subacute toxicity of antimicrobial secondary metabolites of endophytic fungi of *Synclisia scabrida* Miers using rat model.

Methods: Acute oral toxicity was determined following Lork's method. Sub-acute toxicity study was evaluated by administering 200 and 400 mg/kg body weight of the fungal metabolites to rats orally as a single dose for three days, while the control group received normal saline (0.5 mL). The effects of the metabolites on haematological and biochemical parameters were determined using standard methods. The histopathology of the kidney, liver and heart was observed.

Results: The acute oral toxicity studies showed that S1, S2, and R1 metabolites were safe above 5000 mg/kg bodyweight within 24 h. The LD50 of L1 metabolite was below 1000 mg/kg bodyweight. Above 1000 mg/kg bodyweight, the rats given L1 metabolite showed major physical changes, which included initial sluggishness and hyperactivity, resulting in the death of the rats within 20 min of administration. The metabolites showed significant differences ($p = 0.0001$) in means of the baselines and after-treatment haematological parameters at the administered doses. There were no significant changes ($p = 0.05$) in serum ALT, AST, ALP, and creatinine levels of rats. The urea and total protein increased considerably ($p = 0.002$ and 0.000 , respectively) on administration of the metabolites to the rats at the dose levels. The histopathological examination showed varying degrees of degeneration and necrosis of renal tubular epithelial cells and hepatocytes with normal myocardial histo-architecture.

Conclusion: Majority of secondary metabolites of endophytic fungi of *Synclisia scabrida* Miers (S1, S2 and R1 metabolites) caused no death of the rats, even at the highest doses tested, indicating the safety of the metabolites. However, these metabolites should be used at moderate doses as high doses may cause mild organ toxicity.

Keywords: Endophytic fungi, Metabolites, Acute and sub-chronic toxicity, *Synclisia scabrida* Miers

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INTRODUCTION

Due to the problem of antibiotic resistance by pathogenic microorganisms and the emergence

of infectious diseases, there is an urgent need to search for alternative sources of bioactive compounds of medicinal/pharmaceutical interest. Plants have been used for centuries in treating

diseases due to their safety, effectiveness, affordability, and low cost [1]. Endophytes are a reliable potential source of novel compounds with appreciable biological and pharmacological effects. They are microorganisms, bacteria and fungi that inhabit the living tissues of plants without negatively impacting the host plant [2]. They symbiotically associate with the host plant and protect it by competing with pathogens for nutrients, producing antibiotics, and inducing host resistance to biotic and abiotic stresses [3].

Endophytic fungi are diverse and essential microbial flora that produce secondary metabolites, which can serve as rich sources of biologically active compounds. They contribute significantly to the defense mechanisms of their host plant by producing toxic substances such as alkaloids, flavonoids, terpenoids, phenolic compounds, isocoumarins, quinones, steroids, cyanogenic glycosides, strychnine, and digitoxin with potential negative impacts on humans and animals and, therefore, require investigation before their therapeutic usage can be assured [4]. These secondary metabolite-based phyto-compounds elicit a wide range of biological activities, including but not limited to antifungal, antibacterial, antioxidant, immunomodulatory, antiparasitic, anticancer, and antiprotozoal effects [5].

Synclisia scabrida Miers (goat weed) is a shrub traditionally used in tropical Africa, including Nigeria, for treating diseases such as malaria, gastric ulcers, gastrointestinal disorders, dyspepsia, menstrual pains, and sexually transmitted diseases. Different parts of the plant (roots, stem, and leaves) have been reported to possess medicinal properties and to exert different pharmacological effects [6]. The plant parts have been shown to harbour diverse endophytic fungi with inherent antimicrobial activities [5]. There is, however, no documented evidence for its safety or toxicity. The aim of this study, therefore, was to evaluate the acute and sub-acute effects of antimicrobial secondary metabolites extracted from endophytic fungi isolated from *Synclisia scabrida* Miers.

EXPERIMENTAL

Plant collection, isolation, and extraction of bioactive secondary metabolites

Healthy, mature plant parts of *S. scabrida* Miers (leaves, roots, and stems) were collected from Orba in the Udenu Local Government Area of Enugu State, Nigeria, in November 2022. The identity of the plant was certified by Mr. Alfred O. Ozioko, a botanist at International Centre for

Ethnomedicine and Drug Development (InterCEDD), with Voucher number: InterCEDD/700. Endophytic fungi were isolated from different parts of the plant (leaves, stems and roots) following the method described by Okezie *et al* [6]. The resulting four fungal isolates were fermented on rice broth according to Kumar & Kaushik [7] and the corresponding secondary metabolites (S2, S1, R1, and L1) were extracted using ethyl acetate and concentrated at 50 °C under reduced pressure using a rotary evaporator as described by Ozioko *et al* [5].

Animals

Ninety-three (93) healthy adult Wistar rats of both sexes, weighing approximately 60 to 105 g, were used for the study. They were purchased from the animal house facilities of the Pharmacology and Toxicology Department at the University of Nigeria, Nsukka. The animals were provided with sufficient grower's mash feed (Vital Feeds, Nigeria) and tap water *ad libitum* for seven days before the study began. The animals were housed in a standard laboratory environment and allowed to acclimate to the laboratory conditions (27 ± 2 °C) before the study. Animal handling complied with the Guide for Care and Use of Laboratory Animals, 8th Edition, 2011 guidelines [8]. The animals were euthanized by administering 1 mL of ketamine intramuscularly following the University of Nigeria Guidelines on Euthanasia, with efforts made to minimize suffering.

Ethical approval

Ethical approval (no. FRSRA/UNN/23/0084) for the use of animals was obtained from the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka and the handling of animals complied with the relevant laws and institutional and governmental policies guiding research involving animals as enunciated by the National Code of Conduct for Animal Research Ethics (NCARE).

Acute oral toxicity studies

Fifteen (15) healthy adult Wistar rats of both sexes were weighed. The acute oral toxicity (LD₅₀) was determined following Lorke's method [9]. This was carried out in two phases. In the first phase, nine (9) animals were divided into three groups of three animals each. Each group of three animals was administered a single dose of 10, 100, and 1000 mg/kg using a gavage needle. The animals were observed for any sign of toxicity and possible death within 24 hours of treatment. For the second phase, six (6) animals

were divided into three groups of two animals each and were orally administered 1600, 2900, and 5000 mg/kg doses of the fungal metabolites, respectively and were observed for 24 hours for signs of toxicity, after which the final LD₅₀ was determined. Thereafter, the animals were sacrificed.

Effects of fungal metabolites on haematological and biochemical parameters

Blood samples were collected using heparinised hematocrit capillary tubes before and after drug administration through the retro-orbital routes. They were placed in appropriately labelled plain and ethylene diamine tetraacetic acid (EDTA) anticoagulated bottles for biochemical and haematological analysis. The blood samples in plain bottles were centrifuged at 3600 rpm for 5 min to obtain the serum samples.

The haematological parameters, which included serum haemoglobin concentration, red blood cell count, packed cell volume, white blood cell, neutrophil and lymphocyte counts of the control and treated groups, were determined and compared with a control group using a haematological autoanalyzer Mindray BC-2800.

The biochemical analysis was done using the serum obtained by allowing the blood samples without EDTA to clot. Then, they were centrifuged at 2500 r/min for 15 min and stored at -20 °C. Commercial kits from Randox Laboratories Limited, United Kingdom, and Agappe Diagnostics (Switzerland) were, respectively, used to assay the liver and kidney indices according to the manufacturer's instructions.

Histopathological examination

Following blood collection, the animals were sacrificed by injection of 1 mL of ketamine

intramuscularly. The liver, kidney and heart of the rats from each group were excised and preserved in 10% buffered formalin. The tissues were dehydrated using 100 % ethanol and cleared in toluene to increase their refractive index. Processes of infiltration and embedding were carried out using liquid paraffin and molten paraffin wax, respectively, using an L-shaped mould. Sections of the tissues were made using a rotary microtome and stained using hematoxylin and eosin. Thereafter, the histomorphological characteristics of the tissues were examined using a standard binocular light microscope with an in-built Moticam Images Plus 2.0 digital camera (Motic China Group Ltd, China) attached to the Leica binocular light microscope.

Statistical analysis

Data collected from biochemical and haematological analyses are expressed as mean $\pm$ SEM. One-way analysis of variance (ANOVA) using SPSS Version 20 was used to test the significance of the differences in means. Post Hoc test (Tukey HSD) was used for multiple comparison tests among groups. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of fungal metabolites on the body weight of rats

To evaluate the effect of metabolites (S2, S2, R1, and L1) on the treated rats, the body weights of the animals were taken before and after treatment with the different fungal metabolites. There was a significant increase in body weight ($p = 0.000$) on administration of 200 and 400 kg/mg doses of fungal metabolites when compared with the baseline (Figure 1).

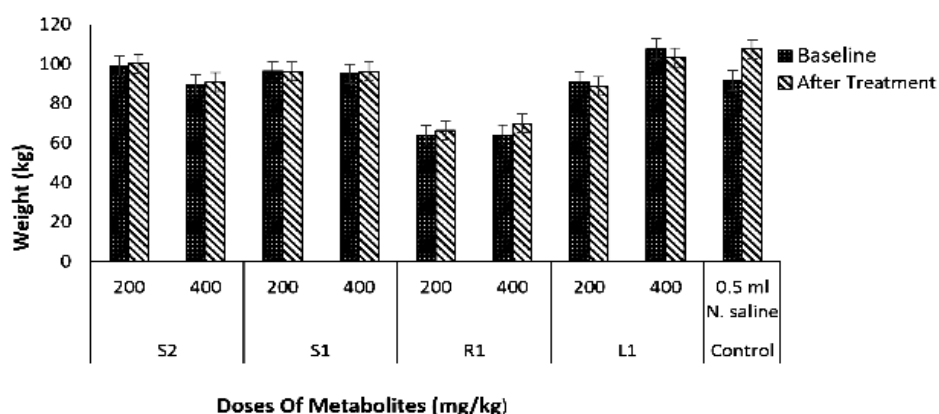


Figure 1: Effect of graded doses of the fungal metabolites on the body weight of rats

Acute oral toxicity

The acute oral toxicity studies showed that none of the rats that received S1, S2 and R1 fungal metabolites showed any significant toxicity effects at the dose level of 1000 mg/kg body weight in the first stage. However, at 2900 and 5000 mg/kg, there were initial dullness,

sluggishness and restlessness, which disappeared within thirty minutes of administration of the metabolites. The L1 fungal metabolite at a 1000 mg/kg dose induced initial sluggishness and hyperactivity, and subsequently led to the death of two rats within 20 minutes of administration (Table 1).

Table 1: Acute oral toxicity studies of crude endophytic fungal metabolites from *S. scabrida* Miers

Group	Dose (mg/kg)	No of deaths	Time of death (min)	% Mortality	Symptoms of toxicity
A: Fungal metabolite from L1 isolate	10	0/3	-	0	No sign of toxicity
	100	0/3	-	0	Sluggish
	1000	2/3	110.5	66.7	Initially hyperactive, then sluggish till death
	Control	0/2	-	0	No symptoms
B: Fungal metabolite from S1 isolate	10	0/3	-	0	No sign of toxicity
	100	0/3	-	0	No symptom
	1000	0/3	-	0	No sign of toxicity
	1600	0/2	-	0	No symptoms
	2900	0/2	-	0	No symptoms
	5000	0/2	-	0	Initial dullness
	Control	0/2	-	0	No symptoms
C: Fungal metabolite from S2 isolate	10	0/3	-	0	No sign of toxicity
	100	0/3	-	0	No symptoms
	1000	0/3	-	0	No toxicity
	1600	0/2	-	0	No symptoms
	2900	0/2	-	0	Initial dullness
	5000	0/2	-	0	Initial restlessness
	Control	0/2	-	0	No symptoms
D: Fungal metabolite from R1 isolate	10	0/3	-	0	No sign of toxicity
	100	0/3	-	0	No symptoms
	1000	0/3	-	0	No changes
	1600	0/2	-	0	No changes
	2900	0/2	-	0	Initial sluggishness
	5000	0/2	-	0	Initial restlessness
	Control	0/2	-	0	No symptoms

Effect of graded doses of fungal metabolites on haematological parameters

Generally, there was a significant difference ($p = 0.0001$) in the baselines and post-treatment values of PCV (%), Hb (g/dL), RBC, and WBC following the administration of different fungal metabolites (Figure 2 to 5). However, no significant difference ($p < 0.005$) was observed in the means of neutrophils and lymphocytes of the animals (Figure 6 and Figure 7). With multiple comparisons, treatment

of the animals with S2 fungal metabolite at 200 mg/kg dose produced a significant increase in the PCV ($p = 0.05$), Hb ($p = 0.033$), and RBC ($p = 0.037$) compared to the baseline. Contrarily, the rise in PCV, HB and RBC levels on administration of the S1 crude drug at a 400 mg/mL dose level was statistically non-significant ($p < 0.05$). Furthermore, treatment with 200 and

400 mg/mL doses of the S1, R1, and L1 fungal metabolites produced significant decreases ($p < 0.05$) in WBC, neutrophil, and lymphocyte levels compared with the baselines.

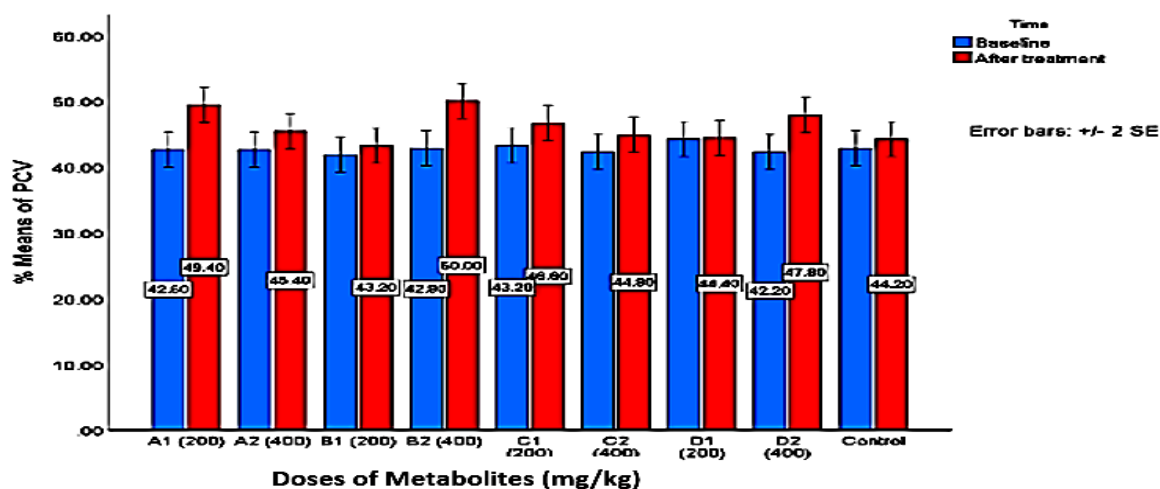


Figure 2: Effect of graded doses of fungal metabolites on the packed cell volume. Where PCV = Packed cell volume; A, B, C and D = S2, S1, R1 and L1 fungal metabolites, respectively; 1 and 2 = 200 and 400 mg/kg of the fungal metabolite, respectively

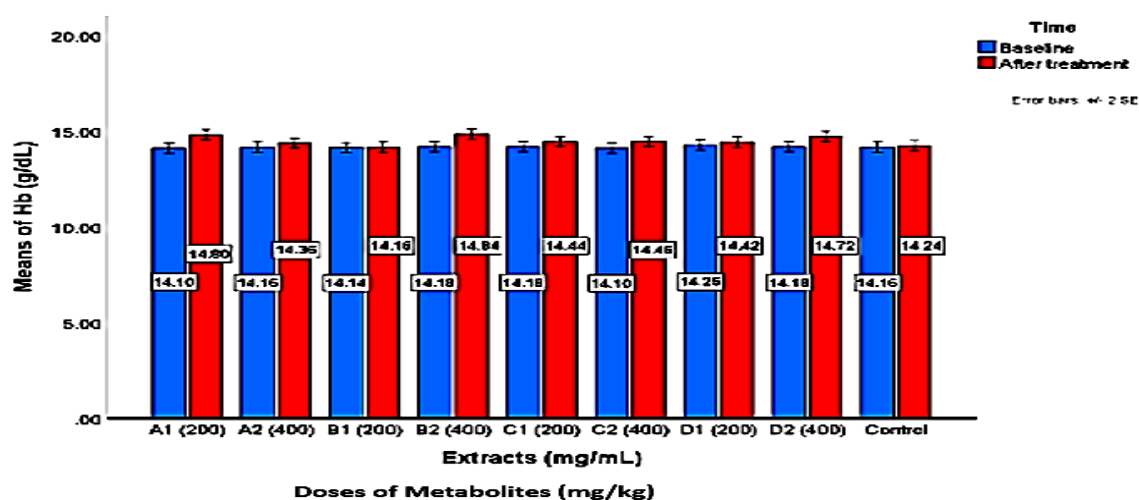


Figure 3: Effect of graded doses of fungal metabolites on the haemoglobin. Where Hb = haemoglobin; A, B, C, and D = S2, S1, R1 and L1 fungal metabolites, respectively; 1 and 2 = 200 and 400 mg/kg of the fungal metabolite, respectively

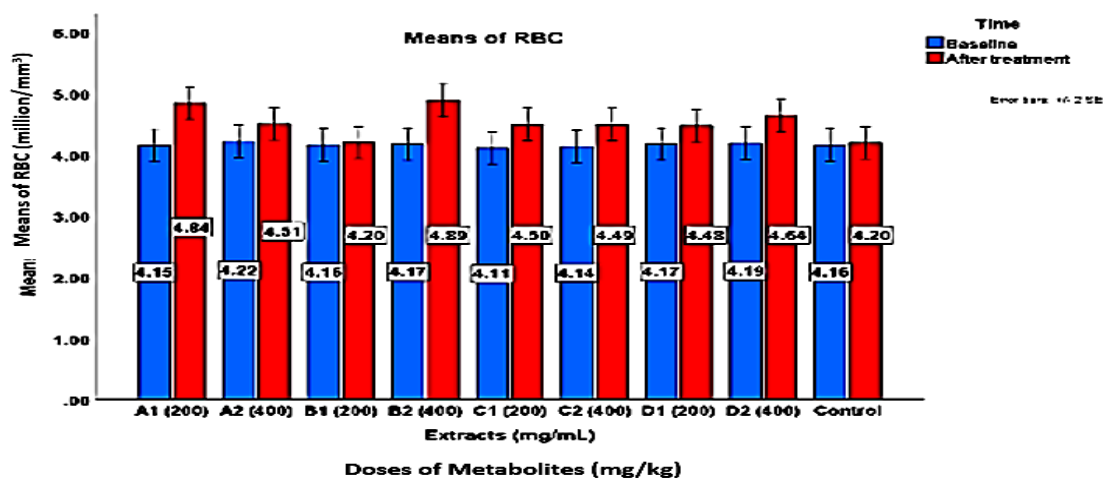


Figure 4: Effect of graded doses of fungal metabolites on the RBC. Where RBC = Red blood cells; A, B, C, and D = S2, S1, R1 and L1 fungal metabolites, respectively. 1 and 2 = 200 and 400 mg/kg of the fungal metabolite, respectively

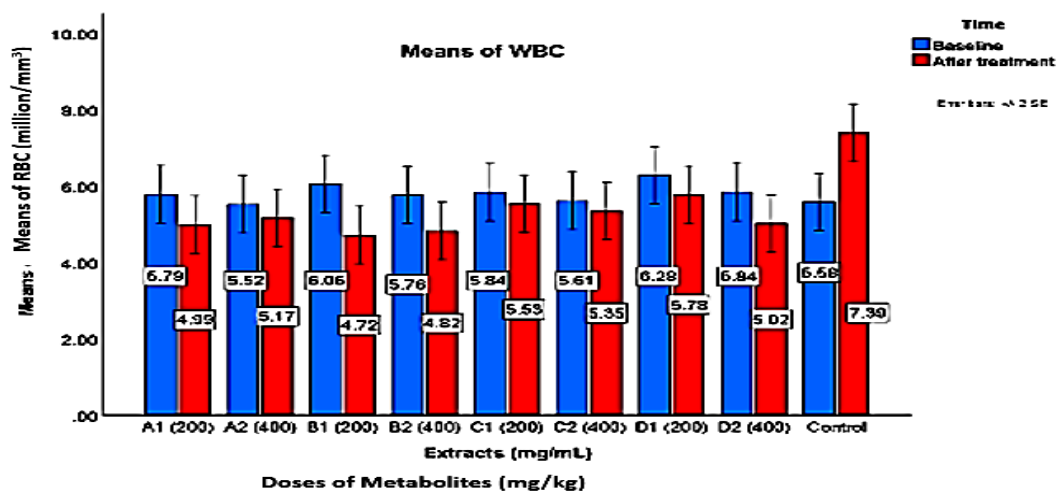


Figure 5: Effect of graded doses of fungal metabolites on the white blood cells. Where WBC = White blood cells; A, B, C, and D = S2, S1, R1 and L1 fungal metabolites, respectively. 1 and 2 = 200 and 400 mg/kg of the fungal metabolite, respectively

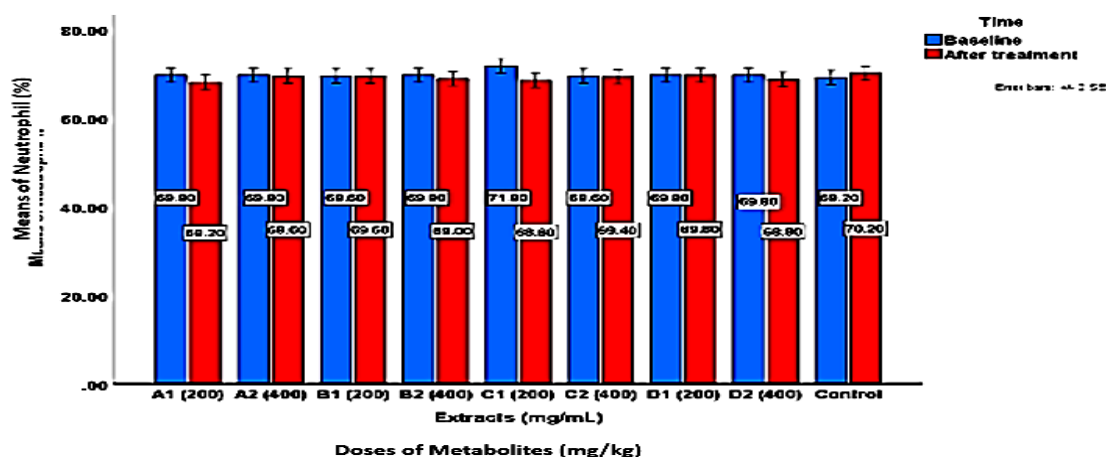


Figure 6: Effect of graded doses of fungal metabolites on the neutrophils. Where A, B, C, and D = S2, S1, R1 and L1 fungal metabolites, respectively, 1 and 2 = 200 and 400 mg/kg of the fungal metabolite, respectively

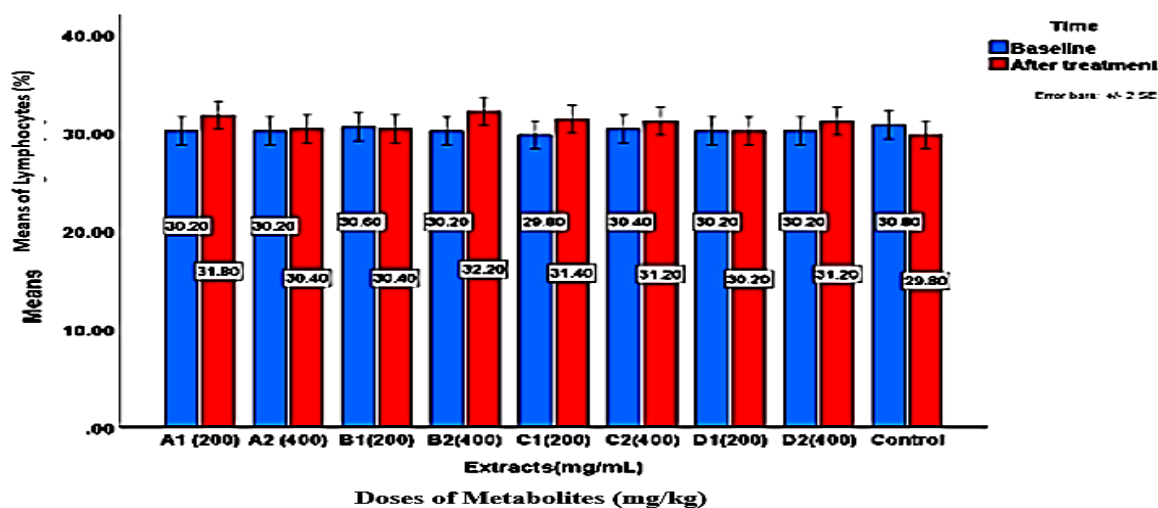


Figure 7: Effect of graded doses of fungal metabolites on the lymphocytes. Where A, B, C, and D = S2, S1, R1 and L1 fungal metabolites, 1 and 2 = 200 and 400 mg/kg of the fungal metabolite, respectively

Effects of graded doses of the fungal metabolites on renal and hepatic markers

Generally, no significant changes ($p = 0.05$) were observed in the levels of ALT, AST, ALP, creatinine and urea in the sera of the animals treated with 200 and 400 mg/kg doses of the different fungal metabolites (Figures 8 to 12). However, there was a significant increase ($p = 0.000$) in the level of total proteins found in the blood of the animals on administration of

metabolites at the two dose levels (Figure 13). With multiple comparisons, when the S1 metabolite was given at 200 mg/kg, there was no significant difference ($p = 0.345$) in the level of TP when compared with the baseline and the control group. There was a significant increase in the level of total proteins on administration of 400 mg/kg of S1 fungal metabolite when compared to the baseline ($p = 0.003$) and the control group ($p = 0.001$).

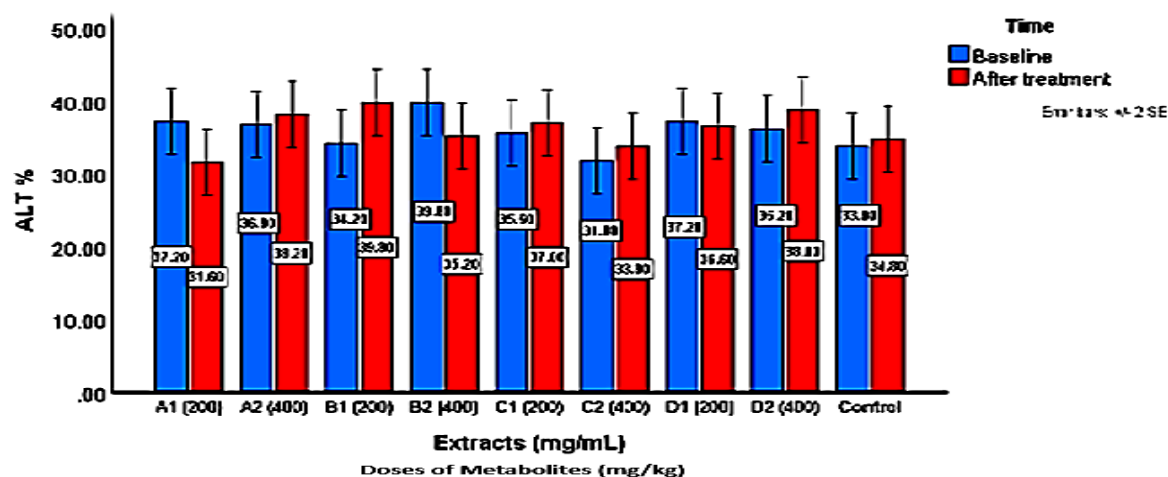


Figure 8: Effects of graded doses of fungal metabolites on alanine transaminase. ALT = Alanine transaminase; A, B, C and D = S2, S1, R1 and L1 fungal metabolites, respectively; 1 and 2 = 200 and 400 mg/kg of the fungal metabolite, respectively

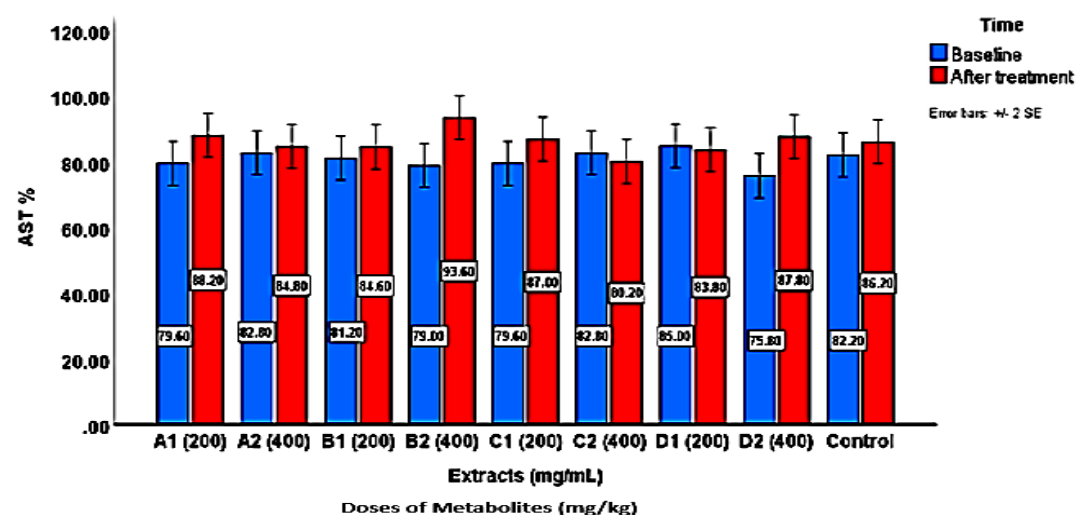


Figure 9: Effects of graded doses of fungal metabolites on the aspartate transaminase. AST = Aspartate transaminase; A, B, C, and D = S2, S1, R1, and L1 fungal metabolites, respectively; 1 and 2 = 200 mg/mL and 400 mg/kg of the fungal metabolite, respectively

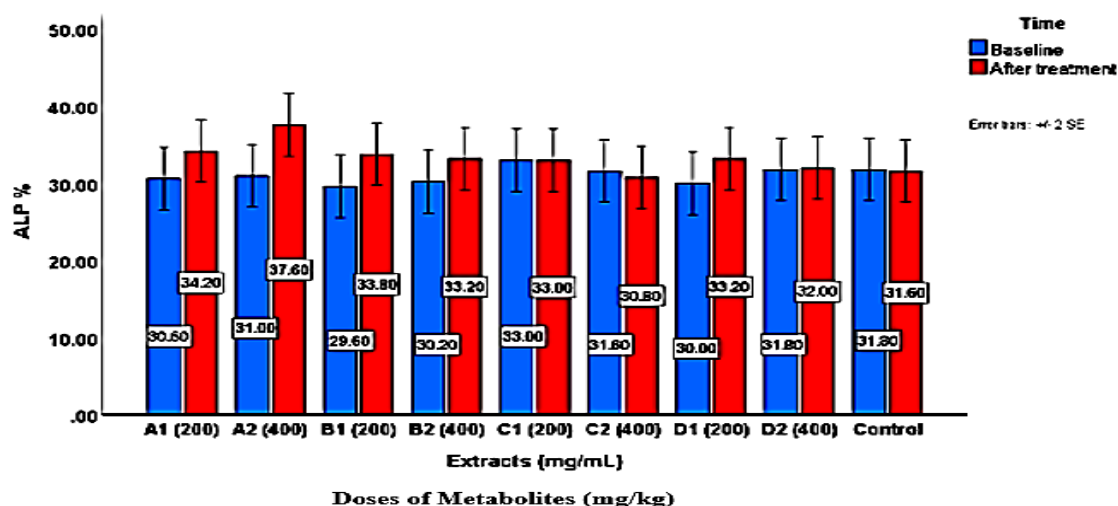


Figure 10: Effects of graded doses of fungal metabolites on the alkaline phosphatase. ALP = Alkaline phosphate; A, B, C and D = S2, S1, R1 and L1 fungal metabolites, respectively; 1 and 2 = 200 and 400 mg/kg of the fungal metabolite, respectively

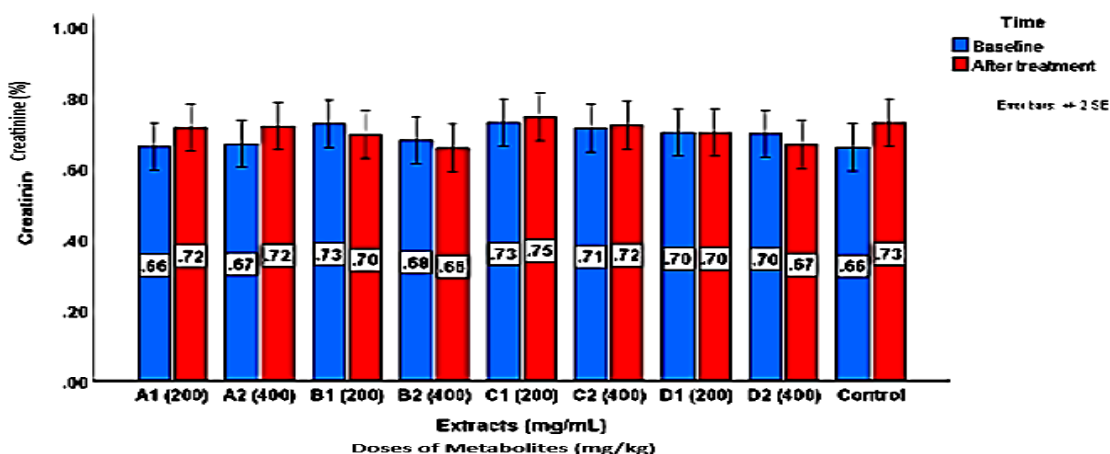


Figure 11: Effects of graded doses of fungal metabolites on the creatinine level. A, B, C, and D = S2, S1, R1, and L1 fungal metabolites, respectively. 1 and 2 = 200 and 400 mg/kg of fungal metabolite, respectively

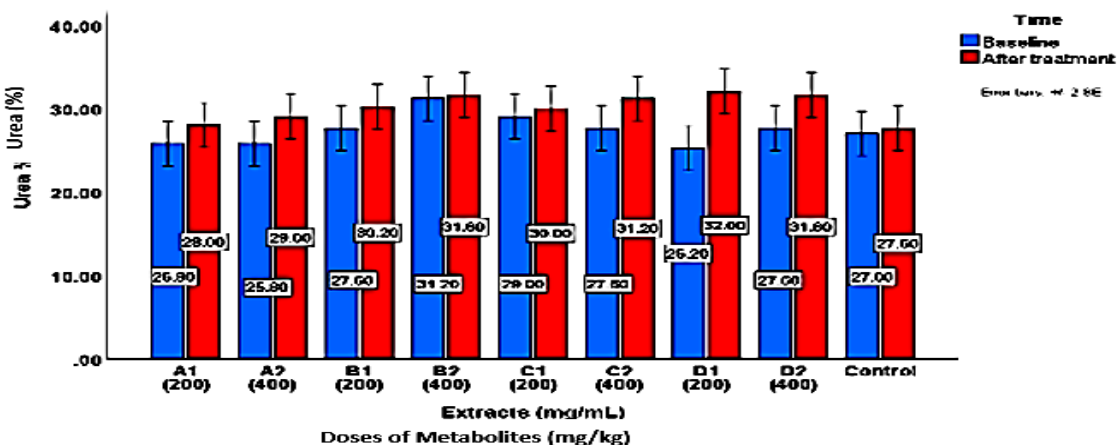


Figure 12: Effects of graded doses of fungal metabolites on the urea. A, B, C, and D = S2, S1, R1, and L1 fungal metabolites, respectively; 1 and 2 = 200 and 400 mg/kg of the fungal metabolite, respectively

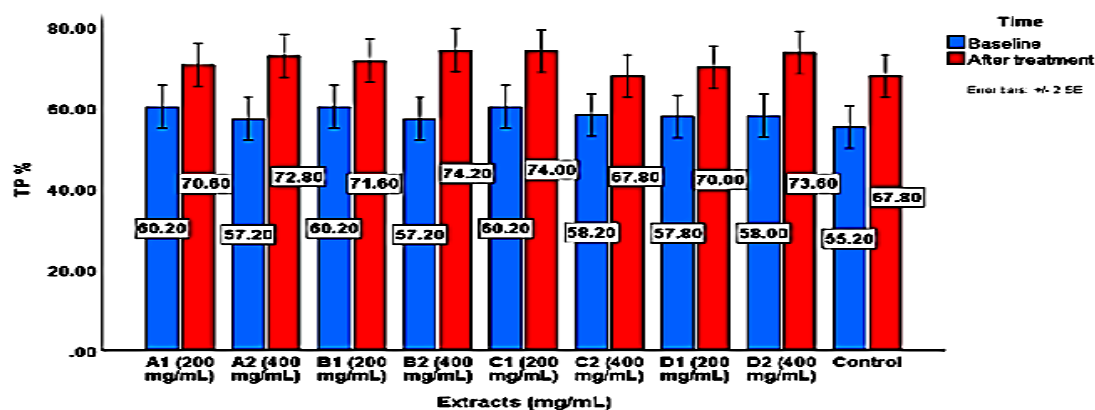


Figure 13: Effects of graded doses of fungal metabolites on the total protein. TP = total protein; A, B, C and D = S2, S1, R1 and L1 fungal metabolites, respectively; 1 and 2 = 200 and 400 mg/kg of the fungal metabolite, respectively

Histopathological findings

The 3-day sub-acute administration of the different doses of the fungal metabolites (200 and 400 mg/kg) showed varying degrees of degeneration and necrosis of renal tubular epithelial cells and hepatocytes with normal myocardial histo-architecture.

The histopathological examination of the kidney sections in rats treated with normal saline and different concentrations of the metabolites is shown in Figures 14 a - d and 15 a - d. The kidneys of rats that received 0.5 normal saline (control) showed mild multifocal vacuolar degeneration and necrosis of the renal tubular epithelial cells in the outer medulla (arrow; Figures 14 e and 15 e). For the rats that received 200 mg/kg body weight, S1 and S2 fungal metabolites showed normal histological features of the glomerulus (G) and renal tubules (arrows) as presented in Figures 14a and b, while R1 and L1 showed mild multifocal vacuolar degeneration and necrosis of the renal tubular epithelial cells in the outer medulla (arrows) as shown in Figures 14 c and d. Sclerosis of the glomeruli (G) was also observed for R1 fungal metabolite (Figure 14 c). At 400 mg/kg body weight, S2 fungal metabolite showed normal renal histological features of the glomerulus (G) and renal tubules (arrows; Figure 15 a). S1, R1, and L1 fungal metabolites showed moderate vacuolar degeneration and necrosis of the renal tubular epithelial cells in the outer medulla (arrow; Figure 15. Sclerosis of the glomeruli (G) was also observed with the S1 metabolite (Figure 15 b).

The death of two rats and other behavioural changes observed in acute oral toxicity of L1 fungal metabolite (Table 1) indicate the toxic effect of the fungal metabolite.

Figure 16 shows the histopathological examination of liver sections of rats treated with normal saline (control) and 200 mg/kg body weight of the different fungal metabolites. S1 and S2 fungal metabolites with the Control showed normal hepatic histo-architecture as presented in Figures 16 a, b, and e. While R1 fungal metabolite showed severe widespread areas of hepatocellular necrosis and periportal inflammatory cellular infiltration and piecemeal necrosis (arrow; Figure 16 c, L1 produced a severe, diffuse vacuolar degeneration and necrosis of the hepatocytes (arrow) and mild to moderate periportal infiltration of inflammatory cells (red arrow; Figure 16 d). For the rats that received 400 mg/kg body weight, S2, R1, and L1 fungal metabolites and control showed normal hepatic histo-architecture (Figure 17 a, c, and e). S1 fungal extract showed moderate periportal inflammatory cellular infiltration and necrosis (arrow; Figure 17 d).

The histopathological examination of heart sections in rats treated with normal saline (control) and 200 and 400 mg/kg body weight of the different fungal metabolites, as presented in Figure 18 a - d and 19 a - d, showed normal myocardial histo-architecture, thus showing that the endophytic metabolites were not toxic to heart tissues.

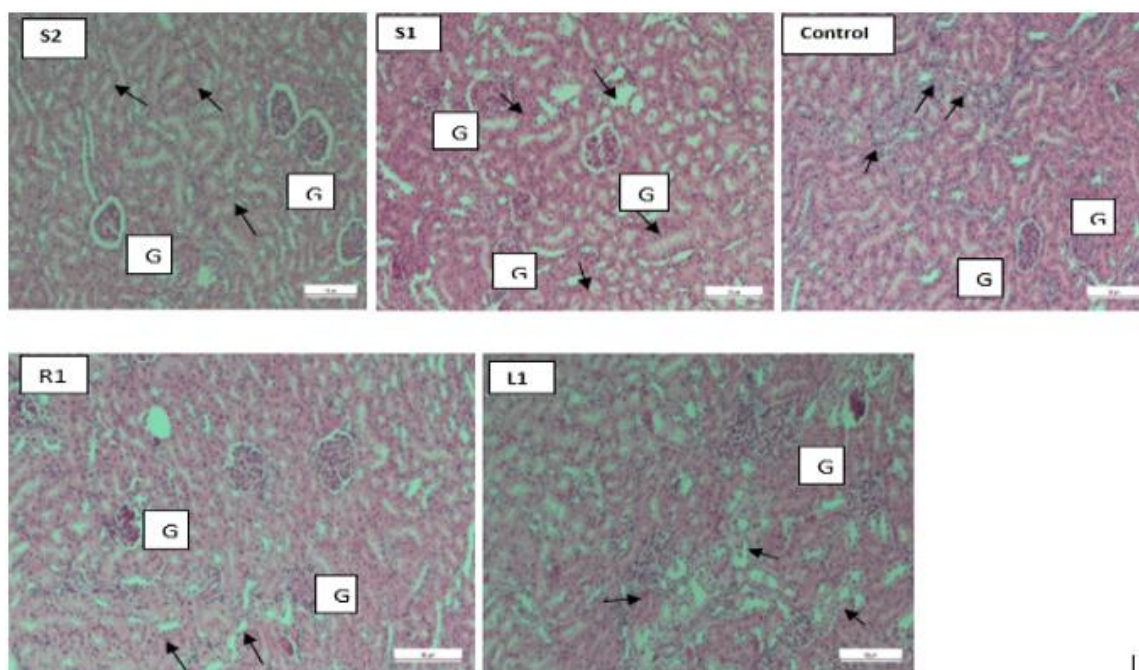


Figure 14: Photomicrographs of kidneys of rats treated with different endophytic fungi metabolites (200 mg/kg). Magnification: $\times 400$. S1 and S2 fungal metabolites showed normal histological features of the glomerulus (G) and renal tubules (arrows). R1, L1 and control showed mild multifocal vacuolar degeneration and necrosis of the renal tubular epithelial cells in the outer medulla (arrow). sclerosis of the Glomeruli (G) was also observed for R1 fungal metabolite

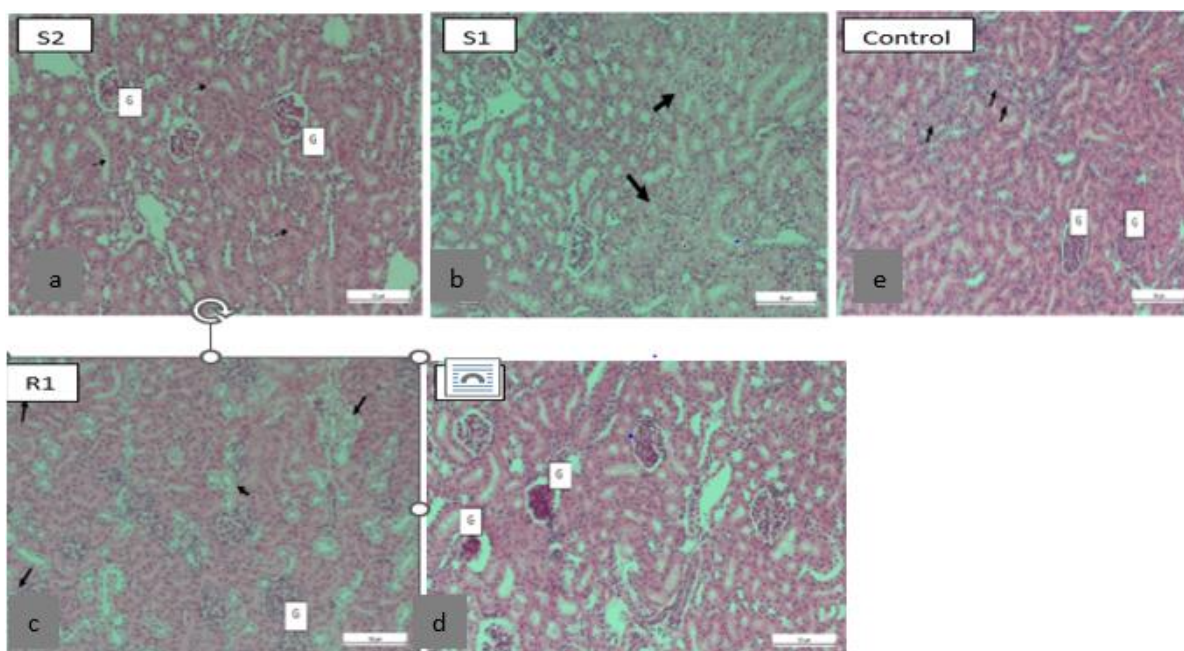


Figure 15: Photomicrographs of rats treated with different endophytic fungal metabolites (400 mg/kg). Magnification: $\times 400$. S2 fungal metabolite showing normal renal histological features of the glomerulus (G) and renal tubules (arrows). S1, R1, L1 and control showed moderate vacuolar degeneration and necrosis of the renal tubular epithelial cells in the outer medulla (arrow). sclerosis of the Glomeruli (G) was also observed in the S1 metabolite

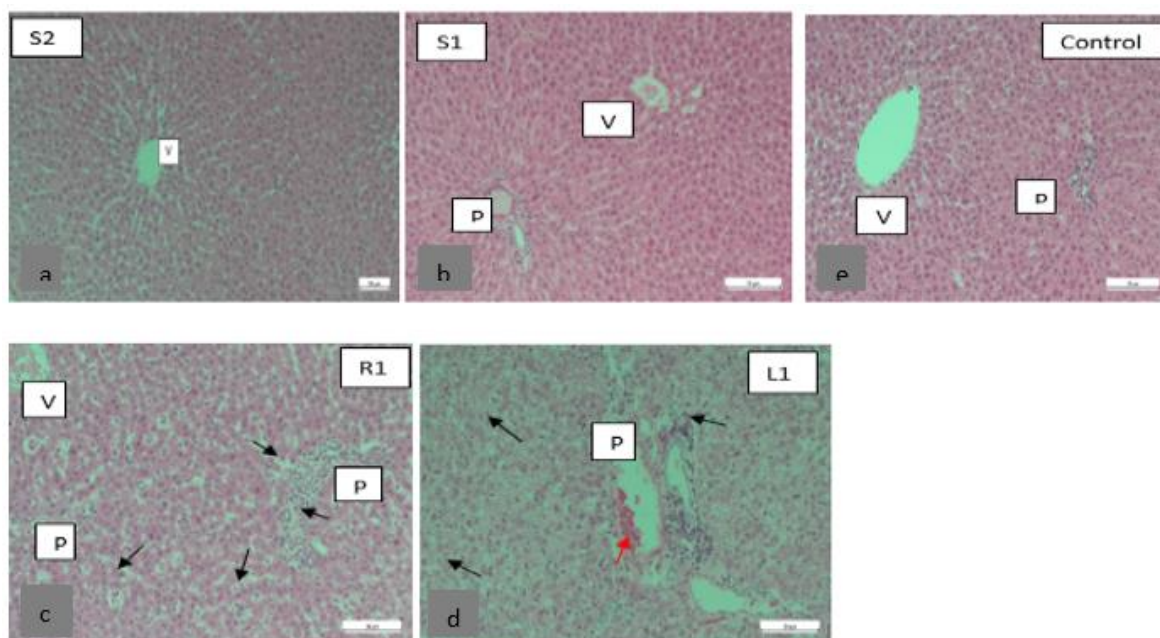


Figure 16: Photomicrographs of the liver of rats treated with different endophytic fungi metabolites (200 mg/kg). Magnification: $\times 400$. S1 and S2 fungal metabolites, with the control, showed normal hepatic histo-architecture. Central vein (V), Portal area (P). R1 showed severe, widespread areas of hepatocellular necrosis and periportal inflammatory cellular infiltration and piecemeal necrosis (arrow). Central vein (V); Portal area (P). L1 showed severe, diffuse vacuolar degeneration and necrosis of the hepatocytes (arrow) and mild to moderate periportal infiltration of inflammatory cells (red arrow). Portal area (P)

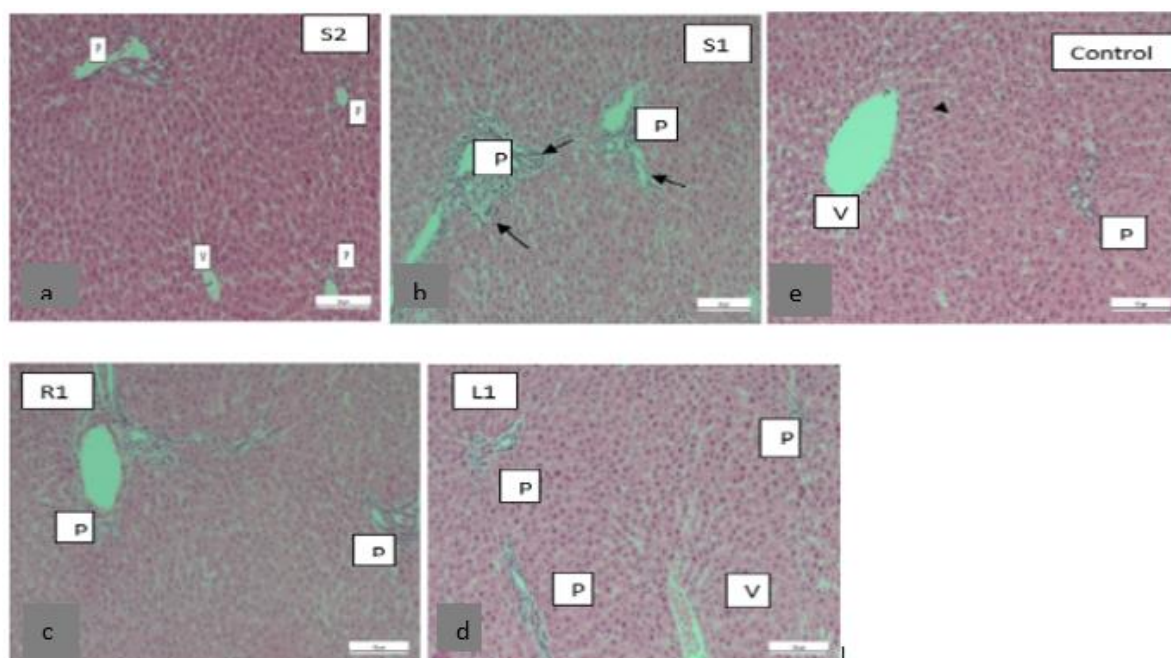


Figure 17: Photomicrograph of the liver of rats treated with different endophytic fungi metabolites (400 mg/kg). Magnification: $\times 400$. S2, R1, and L1 fungal metabolites and control showed normal hepatic histo-architecture. Central vein (V); Portal area (P). S1 fungal extract showed moderately periportal inflammatory cellular infiltration and necrosis (arrow). Portal area (P)

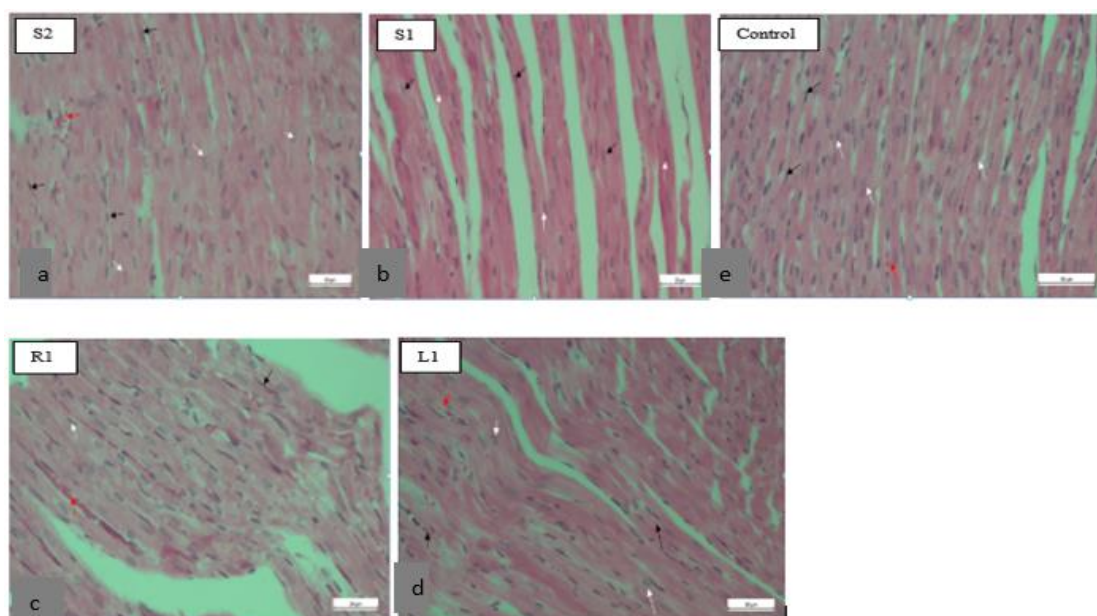


Figure 18: Photomicrographs of the hearts of rats treated with different endophytic fungi metabolites (200 mg/kg). Magnification: $\times 400$. S1, S2, R1, and L1 fungal metabolites and control showed normal myocardial histo-architecture. cardiomyocyte nuclei (white arrow), Pericyte (black arrow), and capillaries (red arrow)

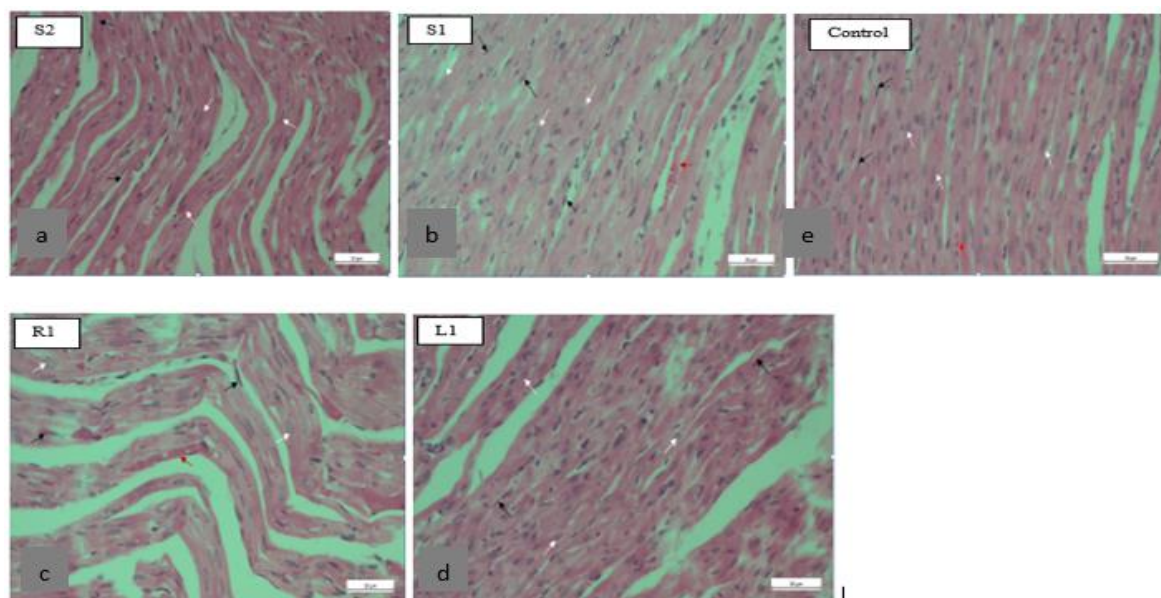


Figure 19: Photomicrographs of the hearts of rats treated with different endophytic fungi metabolites (400 mg/kg). Magnification: $\times 400$. S1, S2, R1, and L1 fungal metabolites with control showed normal myocardial histo-architecture. cardiomyocyte nuclei (white arrow), Pericyte (black arrow), and capillaries (red arrow)

DISCUSSION

The present study investigated the toxicological profiles of secondary metabolites derived from four endophytic fungi isolated from *S. scabrida* Miers. These fungi were previously characterized molecularly and reported to produce secondary metabolites with a range of phytochemicals with antimicrobial activities [5]. Given their therapeutic potential, it was necessary to assess their safety

through acute and sub-chronic toxicity studies in a rodent model.

Endophytic fungi are known to synthesize a wide spectrum of biologically active compounds, including enzymes, antibiotics, polysaccharides, organic acids, vitamins, antioxidants, and various phytochemicals, such as alkaloids, flavonoids, terpenoids, glycosides, and steroids [6,10]. While many of these metabolites have therapeutic properties, others are potentially toxic and have

been linked to carcinogenic, hepatotoxic, nephrotoxic, neurotoxic, and immunosuppressive effects [11]. Hence, comprehensive toxicological profiling remains essential for their safe therapeutic application [12].

The acute oral toxicity evaluation showed that fungal metabolites S1, S2, and R1 were non-lethal at doses up to 5000 mg/kg, consistent with previous reports on endophytes such as *Annona senegalensis* [13]. In contrast, the L1 metabolite induced behavioural changes and mortality at a lower dose (1000 mg/kg), suggesting the presence of toxic phytochemicals like linamarin, a cyanogenic glycoside [5]. Cyanogenic glycosides are hydrolyzed to release hydrogen cyanide (HCN), a potent mitochondrial toxin that disrupts cellular metabolism [14]. These compounds, although protective in plants, pose significant toxicity risks to mammals.

Changes in body weight are a primary indicator of systemic toxicity [15]. In this study, body weight alterations observed were not necessarily indicative of toxicity, as they may reflect physiological adaptation, nutritional variation, or stress responses [16].

Haematological analysis is a critical tool in assessing systemic toxicity and bone marrow function [17]. The study revealed significant changes in PCV, Hb, RBC, and WBC counts across treatments, with S2-treated animals at 200 mg/kg showing significant hematopoietic stimulation. These effects may be linked to specific phytochemicals with immunomodulatory or erythropoietic properties. Conversely, the observed reductions in WBC, neutrophils, and lymphocytes for certain metabolites suggest a potential suppression of immune function, which could have pharmaceutical implications for immunomodulatory drug development.

Biochemical markers such as ALT, AST, ALP, urea, and creatinine provide insight into hepatic and renal function [18]. In general, the fungal metabolites did not cause significant elevations in these markers, suggesting limited hepatotoxic and nephrotoxic effects at the tested doses. However, notable increases in total protein (TP) and urea levels, particularly at higher doses, warrant cautious interpretation. Elevated TP could reflect increased protein synthesis, inflammation, or dehydration, while urea elevation may indicate early renal stress or altered nitrogen metabolism.

Histopathological examination corroborated the biochemical findings. Mild to moderate vacuolar degeneration and necrosis were observed in the

renal tubular epithelium of groups treated with R1 and L1 metabolites, consistent with prior associations between glycosides and tissue necrosis [19]. S1 and S2 metabolites at both 200 and 400 mg/kg generally maintained normal renal and hepatic architecture, with minimal pathological alterations. Severe hepatocellular necrosis and periportal inflammatory infiltrates were notable in rats treated with R1 and L1 metabolites, highlighting their potential for hepatotoxicity.

These observations underscore the importance of evaluating phytochemical constituents beyond their therapeutic promise. It also acknowledges the need for thorough safety assessments [20].

CONCLUSION

The findings of this study show that metabolites from *S. scabrida*-derived endophytic fungi possess diverse biological properties, including antimicrobial and hematopoietic effects. While some are relatively safe with LD₅₀ values, some exhibit signs of nephrotoxicity and hepatotoxicity. These findings support the continued exploration of endophytic fungi as sources of novel bioactive compounds and emphasize the need for rigorous toxicological evaluations.

DECLARATIONS

Acknowledgement/Funding

None.

Use of Artificial intelligence/Large language models

We declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Chizoba A.

Ozioko initiated the project, collected data, and participated in writing the manuscript. Stephen C Emencheta performed statistical analysis; Emmanuel C Ibezim and Anthony A Attama supervised the study. Somtochukwu A Evurani, Dinebari P Berebon, Restus C Onwusoba, and Ezinwanne N Ezeibe reviewed the manuscript. All authors read and approved the final manuscript.

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