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Research Paper

Biochemical and Histomorphological Assessment of Male Rattus Norvegicus after 14 and 28 days of Oral Administration of Ethylacetate Subfraction of Spilanthes Filicaulis

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ABSTRACT

Background: Medicinal plants, including *Spilanthes filicaulis*, have been cornerstone treatments in the traditional primary healthcare system, mainly in tropical African countries. This versatile plant is extensively used to treat and manage various health conditions. In this regard, the present study aimed to amplify scientific evidence about the safety level of *Ethylacetate Subfraction* of *Spilanthes filicaulis* (ESSF).

Methods: A Toxicological study was conducted on 72 male Rattus norvegicus, divided into six groups (n=12) with weight ranges of 140-156 g. Group 1 served as the control group, receiving 10 ml/kg body weight distilled water daily, while Groups 2-6 received graded doses ranging from 62.50 to 750 mg/kg body weight of the extracts, respectively, once daily for 28 days. On days 14 and 28 after the treatment, rats underwent fasting for 12 h overnight and euthanized. Lipid profile and cellular enzymes tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], Gamma-Glutamyltransferase [GGT], Lactate Dehydrogenase [LDH], and Acetylcholine esterase [AchE]) were evaluated on prepared tissues (serum, brain, liver, and kidney). Histoarchitecture of vital organs was also done.

Results: The ESSF at most of the doses investigated on the rats did not significantly alter serum concentrations of lipid on days 14 and 28, compared to the control group ($p>0.05$). Additionally, the ESSF of all doses did not significantly alter the activities of ALT, AST, GGT, LDH, and AChE in most of the studied tissues on days 14 and 28, compared to the control group ($p>0.05$).

Conclusion: No abnormalities were found in the organs of treated animals during the histological analysis. Consequently, *R. norvegicus* treated with ESSF did not experience harmful effects.

Keywords: Cellular enzymes, Lipid profile, Rattus norvegicus, Serum, Tissues

Introduction

Plants are the primary source of medicine for most of the world's population, with approximately 25% of prescribed medicines derived from higher plants [1]. Many traditional herbal remedies have evolved into modern clinical treatments. The popularity of herbal medicine, particularly in rural areas, is attributed to its perceived safety, minimal adverse reactions, and cultural significance, as well as affordable and cost-effective attributes [2]. Medicinal plants contain bioactive compounds that can be toxic if not used properly. Despite the growing popularity of herbal medicine, scientists emphasize the need for rigorous physiological and toxicological testing to ensure safety and efficacy. Therefore, toxicological studies are essential to establish safe doses, prevent harm, and inform clinical applications.

The aster family (Asteraceae) is a vast and diverse group of flowering plants comprising more than 1,600 genera and 23,000 species globally. Despite their vast diversity, members of the aster family share a common chemical profile, most notably a high content of sesquiterpene lactone, which imparts a bitter taste to many of these plants. *Spilanthes filicaulis* is a naturally occurring perennial plant that belongs to Asteraceae. *Spilanthes filicaulis* is characterized by its vibrant yellow flowers and lush green foliage. It is commonly distributed in Africa as well as tropical regions of central and southern America. The leaves are alternate and ovate in shape, with a pungent flavour reminiscent of cress.

Spilanthes filicaulis has been utilized in traditional

medicine for various purposes, including the treatment of malaria, oral health issues, vomiting, and snake bite. Phytochemical screening of crude methanolic leaf extract of *S. filicaulis* has revealed the presence of bioactive principles, such as flavonoids, alkaloids, cardiac glycosides, tannins, steroids, triterpenes, and saponins [3]. This plant possesses a range of bioactive properties, such as antimalarial, antioxidant, anti-inflammatory, and antivenom activities contributing to its therapeutic value [4,5].

Given the remarkable medicinal properties and widespread availability of *S. filicaulis*, it is highly important to investigate its long-term efficacy and safety when consumed daily by indigenous communities. This study aimed to evaluate the toxicity profile of *Ethylacetate Subfraction* of *Spilanthes filicaulis* (ESSF) through 28-day repeated dose toxicity studies through assessment of their effects on lipid profile and cellular enzymes in some of the tissues of male Wistar rats to gain a deeper understanding of the safety profile and potential therapeutic benefits of *S. filicaulis* leaf extract.

Materials and Methods

Plant material

The whole aerial part of the creeping *S. filicaulis* plant utilized in this study was obtained from a farm site located in Igbara Odo Ekiti, Ekiti South West Local Government Area of Ekiti State, Nigeria. This plant was identified by a taxonomist affiliated with the Department of Botany at Obafemi Awolowo University in Ife, Nigeria.

Preparation of *ethylacetate subfraction* of *Spilanthes filicaulis*

Fresh aerial parts of *S. filicaulis* were washed under running laboratory tap water and air dried for a period of two months, which was thereafter ground into a fine powder, and extracted with 80% methanol using a Soxhlet apparatus. The crude methanolic extract was evaporated, and the resulting residue was fractionated into hexane, ethyl acetate, and butanol fractions. Column chromatography of the ethyl-acetate fraction yielded three sub-fractions, designated SFA, SFB, and SFC, after thin-layer chromatographic analysis. These sub-fractions were stored in sealed amber glass jars in the refrigerator, pending further biological evaluation and analysis.

Experimental design

A total of 72 *Rattus norvegicus* with an average weight of 140-156 g were divided into six groups, each comprising 12 rats. Following a seven-day acclimatization period, the rats were administered sub-fraction C extract of *S. filicaulis* orally for 14 and 28 days. The administration volume for all groups of rats was 0.5 ml daily as follows:

Group 1: Control (0.5 distilled water)

Group II: 62.50 mg/kg body weight of SFC extract of *S. filicaulis.*

Group III: 125.0 mg/kg body weight of SFC extract of *S. filicaulis.*

Group IV: 250.0 mg/kg body weight of SFC extract of *S. filicaulis.*

Group V: 500.0 mg/kg body weight of SFC extract of *S. filicaulis.*

Group VI: 750.0 mg/kg body weight of SFC extract of *S. filicaulis*

Sample collection and preparation

Rats were sacrificed 12 h after the last dose on days 14 and 28. Blood samples were collected in plain bottles, centrifuged at 3,000 rpm for 10 min, and the serum was carefully pipetted into another properly labelled plain bottle. Organs (kidney, brain, and liver) were harvested, weighed, and homogenized. Homogenates and blood samples were stored frozen for biochemical analyses.

Determination of lipid profiles

Serum total cholesterol, triacylglycerol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol concentration was estimated according to the method described by Tietz [6] and Friedwald et al. [7].

Determination of cellular enzymes

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), and acetylcholinesterase activities were carried out according to the methods described by Reitman and Frankel [8], Szasz et al. [9], Wroblewski and Ladue [10], and Magnotti et al. [11].

Histopathological examination

Sections of the male rats (kidney, liver, and brain) were preserved in 10% mild buffered formalin by complete immersion for 24 h, shortly after which they were cut to around 3-5mm thick pieces and processed using the wax made from paraffin-embedded method. Using a rotary microtome, tissue blocks were cut into sections that were 5 µm thick. Tissue sections were stained with hematoxylin and eosin for histological analysis. Morphological changes were then examined using a light microscope [12].

Statistical analysis

Data were analysed using one-way analysis of variance followed by Duncan's post-hoc test ($p<0.05$). Results are presented as mean ± standard error of the mean. Statistical significance was at a 95% confidence level. Data visualization was performed using GraphPad Prism Software.

Results

Lipid profile indices

The fraction C of *Spilanthes filicaulis* ethyl acetate partition extract at all doses investigated on the rats after 28

days of oral administration did not significantly alter the serum concentrations of triglycerides on days 14 and 28, compared to the control groups (p>0.05). However, the concentrations of total cholesterol slightly increased at doses of 62.50 and 500 mg/kg body weight after 28 days of oral administration in comparison to the other groups (p<0.05). Conversely, the concentrations of LDL cholesterol decreased (p<0.05) after 28 days of oral administration at most of the doses, compared to 14 days, with the reduction even more pronounced at the highest dose of 750 mg/kg body weight. In addition, a slight increase (p<0.05) was observed for the LDL cholesterol concentration at the dose of 62.50 mg/kg body weight, compared to the control. Likewise, a slight increase was observed for the HDL-C concentration at most of the highest doses on days 14 and 28, compared to the control group (p<0.05) (Table 1).

Table 1. Effects of oral administration of fraction C from ethylacetate partitioned extract of *Spilanthes filicaulis* aerial parts on serum lipid profiles of rats on day 14

Treatment	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)	Atherogenic Index
Control	116.38 ± 0.80^{a}	85.72 ± 1.11^{b}	56.79 ± 2.44^{c}	42.44 ± 1.51^{d}	1.05 ± 0.22^{a}
62.50 mg/kg body weight	118.53 ± 1.25^{a}	86.42 ± 0.62^{b}	57.51 ± 0.73^{c}	43.73 ± 1.72^{d}	1.06 ± 0.45^{a}
125 mg/kg body weight	114.88 ± 2.44^{a}	85.49 ± 2.53^{b}	58.62 ± 1.48^{c}	39.17 ± 2.92^{d}	0.96 ± 0.19^{a}
250 mg/kg body weight	112.83 ± 5.30^{b}	82.45 ± 1.45^{b}	55.98 ± 0.77^{c}	40.35 ± 5.43^{d}	1.02 ± 0.28^{a}
500 mg/kg body weight	117.55 ± 0.52^{a}	88.91 ± 1.39^{b}	58.07 ± 2.23^{c}	41.69 ± 2.69^{d}	1.03 ± 0.20^{a}
750 mg/kg body weight	117.04 ± 2.51^{a}	80.98 ± 4.11^{c}	62.73 ± 1.23^{d}	38.11 ± 2.41^{d}	0.87 ± 0.14^{b}

Values are means ± standard error of mean of six replicates. Values in the identical column with different superscripts are significantly different (p<0.05).
HDL: high-density lipoprotein; LDL: low-density lipoprotein

Cellular enzymes

Alanine aminotransferase activity

The fraction C of *Spilanthes filicaulis* ethyl acetate partition extract at all doses investigated on the rats after 28 days of oral administration did not significantly modify the ALT activities in the serum and liver on days 14 and 28, compared to the control group (p>0.05) (Figure 1).

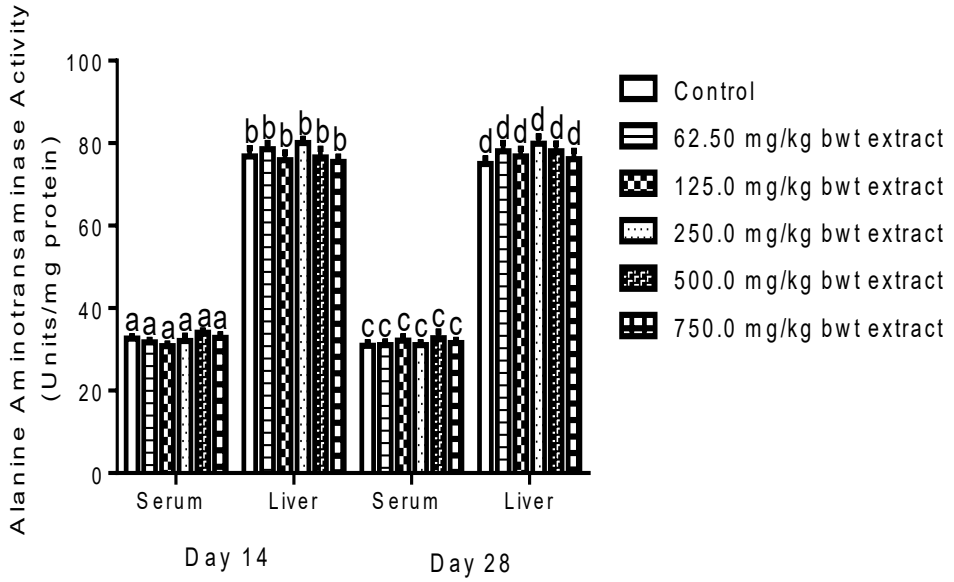

Figure 1. Effects of oral administration of fraction C from *ethylacetate subfraction* of *Spilanthes filicaulis* aerial parts on specific activity of alanine aminotransferase in serum and *Rattus norvegicus* liver on days 14 and 28. Values are means ± standard error of mean of six replicates. Bars with similar alphabets are not too different (p>0.05)

Aspartate aminotransferase activity

The fraction C of *Spilanthes filicaulis* ethyl acetate partition extract at all doses investigated on the rats after 28 days of oral administration did not significantly modify the AST activities in the serum and liver on days 14 and 28, compared to the control group (p>0.05) (Figure 2).

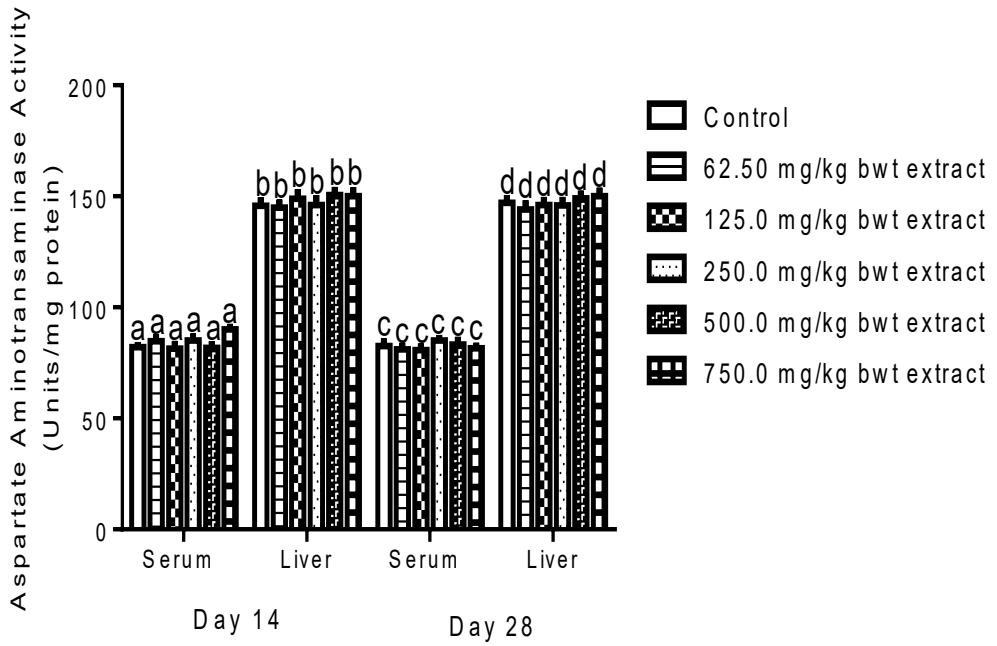

Figure 2. Effects of oral administration of fraction C from *ethylacetate subfraction* of *Spilanthes filicaulis* aerial part on specific activity of aspartate aminotransferase in Serum and *Rattus norvegicus* liver on days 14 and 28. Values are means ± standard error of mean of six replicates. Bars with similar alphabets are not too different (p>0.05)

Gamma-glutamyltransferase activity

The fraction C of *Spilanthes filicaulis* ethyl acetate partition extract at all doses investigated on the rats after 28 days of oral administration did not significantly modify the GGT activities in the serum, kidneys, and liver on days 14 and 28, compared to the control group (p>0.05) (Figure 3).

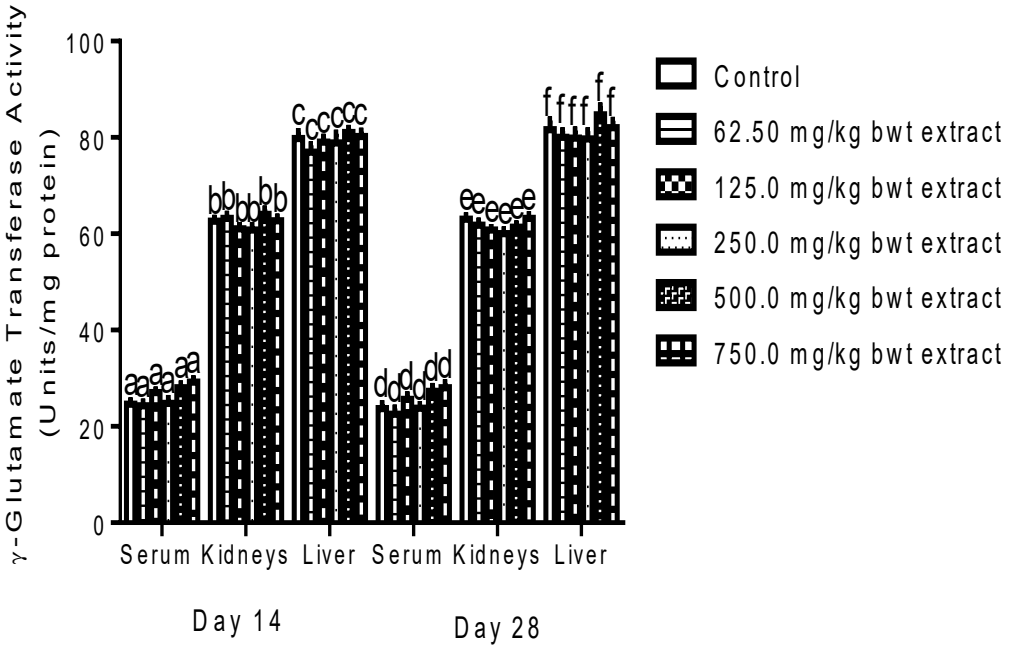

Figure 3. Effects of oral administration of fraction C from *ethylacetate subfraction* of *Spilanthes filicaulis* aerial part on specific activity of gamma-glutamyltransferase in serum, kidneys, and liver of *Rattus norvegicus* on Days 14 and 28. Values are means ± standard error of mean of six replicates. Bars with similar alphabets are not too different (p>0.05)

Lactate dehydrogenase activity

The fraction C of *Spilanthes filicaulis* ethyl acetate partition extract at all doses investigated on the rats after 28 days of oral administration did not significantly alter the activities of LDH in the serum and liver on days 14 and 28, compared to the control group (p>0.05) (Figure 4).

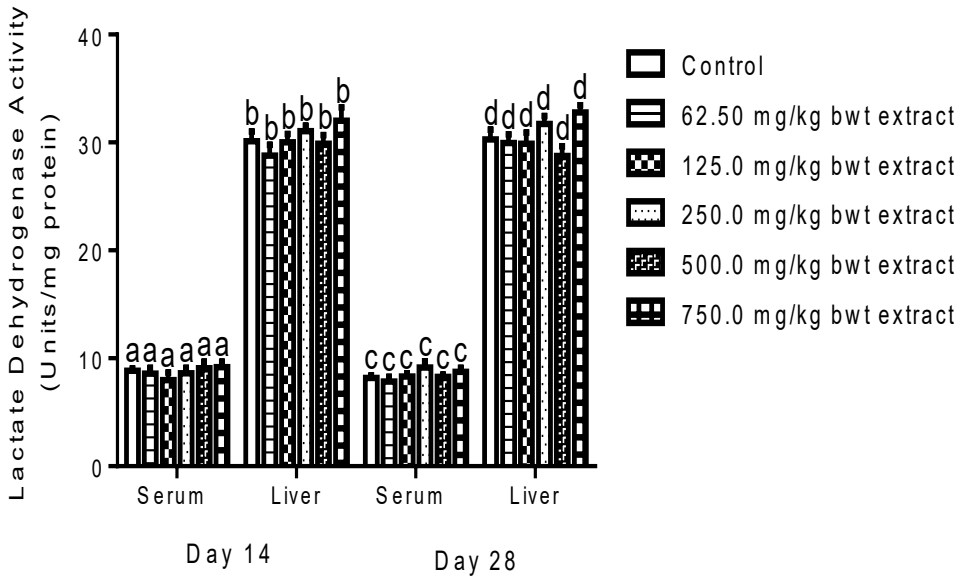

Figure 4. Effects of oral administration of fraction C from *ethylacetate subfraction* of *Spilanthes filicaulis* aerial parts on specific activity of lactate dehydrogenase in serum and liver of *Rattus norvegicus* on Days 14 and 28. Values are means ± standard error of mean of six replicates. Bars with similar alphabets are not too different (p>0.05)

Acetyl cholinesterase activity

The fraction C of *Spilanthes filicaulis* ethyl acetate partition extract at all doses investigated on the rats after 28 days of oral administration did not significantly alter the activities of Acetylcholinesterase (AChE) in the brain on days 14 and 28, compared to the control group (p>0.05) (Figure 5).

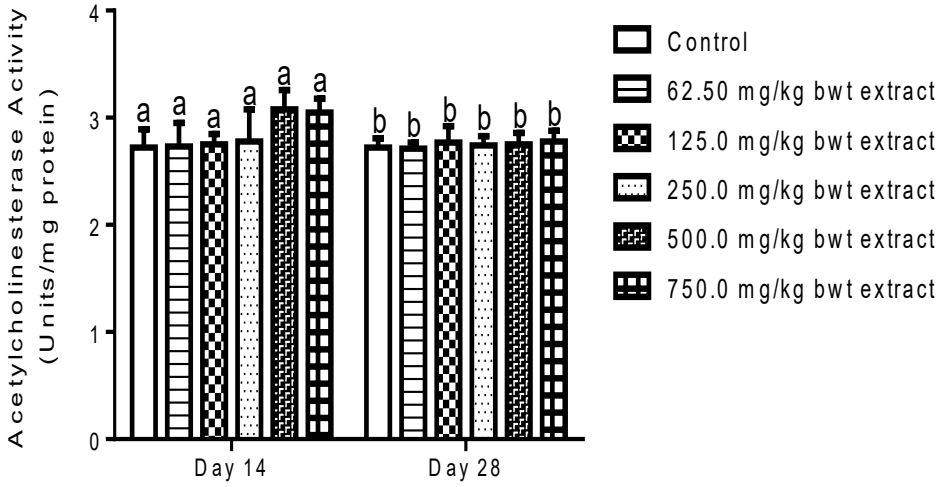

Figure 5. Effects of oral administration of fraction C of *ethylacetate subfraction* of *Spilanthes filicaulis* aerial part on specific activity of acetylcholinesterase in the brain of *Rattus norvegicus* on Days 14 and 28. Values are means ± standard error of mean of six replicates. Bars with similar alphabets are not too different (p>0.05)

Discussion

For centuries, humans have relied on plant-based products for disease treatment and management, often without prior understanding of potential adverse effects [13]. However, growing evidence suggests that improper use of herbal products can lead to severe health complications, including liver and kidney damage [14]. Given these concerns, the present study aimed to investigate the biochemical and histological effects of oral administration of ESSF in male *R. norvegicus*.

Lipid profile is the breakdown of the components of fats, such as triacylglycerol, total cholesterol, LDL cholesterol, and HDL cholesterol, found in blood. An abnormal proportion of any of the class of these fats increases the risk of cardiovascular diseases [15]. In the present study, the oral administration of SFC extracts after 28 days produced a normal threshold limit of serum total cholesterol and triglyceride activity, which were significantly not altered at most doses when compared to the control group (Tables 1 and 2). However, a slight reduction in activity was observed on day 28, compared to day 14, at the graded doses in serum cholesterol and triglyceride may be due to the reduced intake through the intestines, facilitating its interaction with intestinal bile acid and boosting bile acid secretionet [16]. Additionally,

the presence of saponin phytoconstituents has been able to produce compounds that are insoluble with their progenitor bile salt, consequently, making them unavailable for absorption [17].

Table 2. Effects of oral administration of fraction C from ethylacetate partitioned extract of *Spilanthes filicaulis* aerial parts on serum lipid profiles of rats on day 28

Treatment	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)	Atherogenic Index
Control	112.51 ± 1.82^{a}	79.40 ± 1.15^{b}	57.47 ± 1.68^{c}	39.17 ± 3.29^{d}	0.96 ± 0.18^{a}
62.50 mg/kg body weight	119.79 ± 0.88^{b}	78.93 ± 0.77^{b}	58.47 ± 0.95^{c}	45.54 ± 0.58^{e}	1.08 ± 0.37^{a}
125 mg/kg body weight	112.64 ± 4.54^{a}	78.79 ± 0.92^{b}	59.69 ± 1.35^{c}	37.95 ± 4.82^{d}	0.89 ± 0.18^{a}
250 mg/kg body weight	110.29 ± 7.03^{a}	79.91 ± 0.76^{b}	59.08 ± 1.42^{c}	35.24 ± 7.61^{f}	0.87 ± 0.12^{a}
500 mg/kg body weight	117.36 ± 1.32^{b}	81.53 ± 1.12^{b}	61.82 ± 0.39^{d}	39.24± 1.45^{e}	0.89 ± 0.08^{a}
750 mg/kg body weight	113.89 ± 4.17^{a}	78.96 ± 1.79^{b}	63.74 ± 1.05^{d}	34.36 ± 3.54^{f}	0.79 ± 0.16^{a}

Values are means ± standard error of mean of six replicates. Values in the identical column with different superscripts are significantly different (p<0.05). HDL: high-density lipoprotein; LDL: low-density lipoprotein

The LDL cholesterol transports cholesterol from the liver to the intended use site. It carries roughly 60–70% of the total cholesterol; consequently, an increase in total cholesterol activity leads to an increase in LDL cholesterol [18]. An excess of LDL cholesterol activity in the body may initiate the process of atherosclerosis [19]. Serum LDL cholesterol activity in rats was not significantly altered after 14 days of oral administration of SFC at various doses in the serum; however, the reduction was significant after 28 days at the high doses, compared to the control and other dose groups. Consequently, there was a lack of susceptibility to atherosclerosis and other associated cardiovascular disorders (Table 1-2). Atherosclerosis reduces the amount of blood provided by narrowing the capillaries that distribute blood through the channels. Numerous other conditions, including heart attacks, stroke, coronary heart disease, and hypercholesterolemia, can result from an excess of LDL cholesterol [20].

An anti-atherogenic lipoprotein called HDL cholesterol transports cholesterol from peripheral tissues and returns it to the liver, where it is transformed into bile acids [21]. Increased activity of HDL cholesterol observed at the high doses is linked with a healthy heart, thereby reducing risk for related cardiovascular complications, such as myocardial infarction, stroke, and death [22]. Additionally, this might be due to the stimulatory activity of lecithin-cholesterol acyl transferase, an enzyme that transforms free cholesterol into HDL cholesterol [23]. This promotes reverse cholesterol mobility, competitively inhibits LDL cholesterol uptake of endothelial cells, and prevents the production of oxidized LDL cholesterol [24]. Findings of the present study corroborate the observation of Efosa et al. [25], who asserted no significant difference (p>0.05) in the lipid profile (total cholesterol, triglyceride, LDL cholesterol, and HDL cholesterol) of experimental animals compared to the control group when oil from the seed of *Dacroydes edulis* was administered orally to male rats.

The liver is a vital organ in animals receiving the highest amount of blood supply, which accounts for 25% of the total cardiac output [26]. The liver, being the major site of drug metabolism, encounters many foreign substances entering the body. Since the liver performs diverse functions, no single test is sufficient to provide a complete estimate of its functionality [27]. Liver function biomarkers evaluated in this study are a useful screening tool, effectively used to detect hepatic dysfunction, such as aminotransferases (ALT and AST), GGT, and LDH. Enzyme activities of ALT, AST, GGT, and LDH in the serum and liver of rats were not significantly altered at any dose of SFC 14 and 28 days after the oral administration, compared to the control group. This may suggest that 28 days of oral administration of the sample did not cause liver damage, likely did not cause obstruction to the hepatobiliary duct in the hepatocytes, and therefore, did not result in leakage of the enzymes into the circulatory system (Figures 1-4).

The normalization of most of the liver function enzymes of ALT, AST, GGT, and LDH observed may be associated with the membrane maintenance and stabilization of hepatocyte integrity potentials [28], thereby preventing the spillage of liver enzymes into the serum for circulation. Results of the present research are in line with earlier observations made by Obia et al. [29] and Enenebeaku et al. [30], who investigated the extract of *Justicia carnea* in high-fat diet-fed rats and the methanolic, aqueous leaves extract of *Dictyandra arborescens* (Welw.) and *Chasmanthera dependens* (Hochst) on biochemical enhancement activities. Both studies reported no significant elevation in the activities of liver enzymes AST, ALT, and GGT.

The AChE, located on the post-synaptic membrane, helps terminate signal transmission by hydrolyzing acetylcholine to acetate and choline [31]. The AChE activities in the brain were not significantly altered at any dose of SFC after 14 and 28 days of oral administration in rats, compared to the control group. This implies that the sample may not affect cholinergic neurotransmission in the animals (Figure 5).

The kidney is highly susceptible to toxicants due to its high blood supply and its role in filtering large amounts of toxins that may concentrate in the kidney tubules [32]. Toxic substances, such as drugs, heavy metals, chemicals, and immunological complexes, can cause injury to the kidney and, in return, incapacitate it from performing prominent excretory functions that may lead to renal failure [33]. The presence of tissue damage in the kidney can be evaluated by measuring the

serum level of the activity of GGT. Tissue damage results in the release of these confined membrane enzymes into blood bloodstream, resulting in elevated blood levels of such enzymes. The GGT activities in the kidneys and serum at all doses after 28 days of oral administration of SFC on rats compared favourably with the control on days 14 and 28. This finding may suggest that the sample did not induce a distortion of the kidney plasma membrane, which is in line with the findings of Efosa et al. [25].

Results of the biochemical treatment assessing the toxicity of SFC of *S. filicaulis* on rats were supported by the histopathological evaluation of the tissues. In this study, the kidney presents normal renal histomorphology in most of the treatment groups, except for mild alterations, like glomerular atrophy and tubular congestion observed in group 2 after a prolonged oral administration for 28 days (Plate 1). On day 28, liver histomorphology appeared intact in groups 1, 2, 4, and 6 as substantiated by the insignificant changes in ALT, AST, LDH, and GGT (p>0.05). However, central vein and sinusoidal congestion were noticed in groups 3 and 5, which could be suggestive of accumulation after prolonged use (Plate 2). The brain cerebellar cortex after 28 days of oral administration presented a normal architectural histology, compared to the control group (Plate 3).

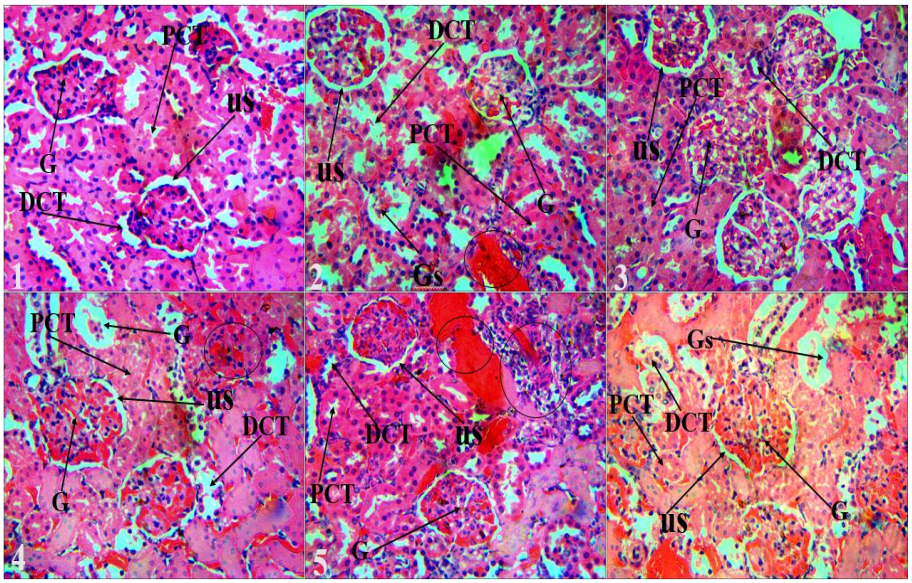

Plate 1 Effects of fraction C of *ethylacetate subfraction* of *Spilanthes filicaulis* on the histoarchitecture of the renal cortex on day 28 (×200, hematoxylin and eosin). Groups 1, 3, 4, 5, and 6 present normal renal histomorphology. Mild features, such as glomerular atrophy (Gs) and tubular congestion (circle), were seen in group 2. 1: Control group (distilled water), 2: 62.50 mg/kg bodyweight extract, 3: 125mg/kg bodyweight extract, 4: 250 mg/kg bodyweight extract, 5: 500 mg/kg bodyweight extract, 6: 750 mg/kg bodyweight extract

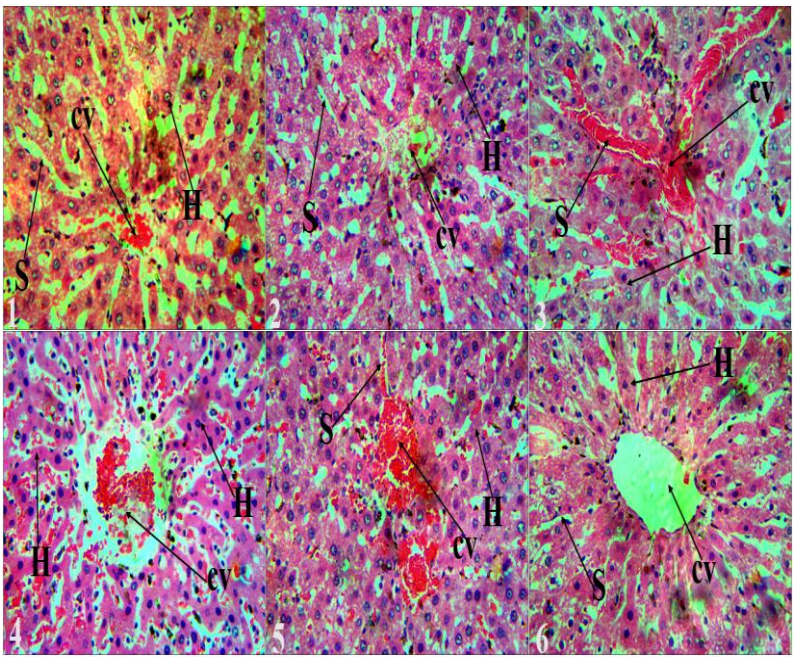

Plate 2 Effects of fraction C of *ethylacetate subfraction* of *Spilanthes filicaulis* on the histoarchitecture of the rat liver on day 28 (×200, hematoxylin and eosin). Liver histomorphology appeared intact in groups 1, 2, 4, and 6. Central vein (CV) and sinusoidal congestion were noticed in groups 3 and 5. S: sinusoid, E: endothelial cell nucleus. 1: Control group (distilled water), 2: 62.50 mg/kg bodyweight extract, 3: 125mg/kg bodyweight extract, 4: 250 mg/kg bodyweight extract, V: 500 mg/kg bodyweight extract, 6: 750 mg/kg bodyweight extract

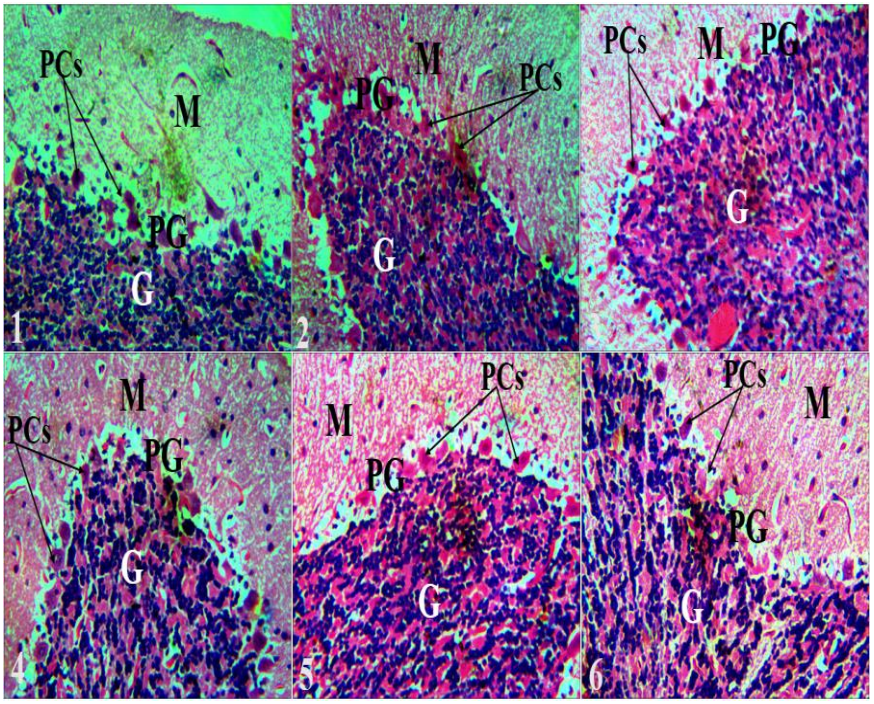

Plate 3 Effects of fraction C of *ethylacetate subfraction* of *Spilanthes filicaulis* on the histoarchitecture section from cerebellum of rats showing its three-toned layers, typical of a normal cerebellum, and the cells of the cortex on day 28 (×200, hematoxylin and eosin). Across the groups, the outer molecular layer has few cells, and the inner granule cell layer (G) is densely packed with cell bodies of small neurons. The middle Purkinje cells (PCs) form a row along the deep margin of the molecular layer, exhibiting their few perikarya; all of which characterize a normal cerebellum. 1: Control group (distilled water), 2: 62.50 mg/kg bodyweight extract, 3: 125mg/kg bodyweight extract, 4: 250 mg/kg bodyweight extract, V: 500 mg/kg bodyweight extract, 6: 750 mg/kg bodyweight extract

Conclusions

Based on the results, it can be said that ESSF did not cause unfavourable changes in biochemical markers, including serum and tissue AST, ALT, GGT, LDH, and lipid profiles. Moreover, no histopathological abnormalities were observed in the liver, kidney, and brain tissues of the test animals. However, to investigate the active ingredients causing this activity and to clarify the potential biochemical process, more research is required in the areas of isolation and characterisation.

Ethical Considerations

Ethical clearance was obtained on 11 May 2023 from the Ethics Committee of the University of Ilorin (UERC Approval Number: UERC/ASN/2023/2475).

Authors' Contributions

All authors contributed to the study design. Conceptualization: B. E. O.; Methodology: B. E. O., O. B. O., O. D. A.; Formal analysis and investigation: B. E. O., O. B. O., O. D. A.; Original draft preparation: B. E. O., O. O. O.; Reviewing and Editing: J. O. O., S. E. A.; Resources: B. E. O., O. B. O., J. O. O., S. E. A., O. D. A., O. O. O., M. G. A.; Supervision: M. G. A. All authors read and approved the manuscript.

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Conflict of Interests

All authors certify no conflict of interests in the content of this study

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Abbreviations

ESSF: *Ethylacetate Subfraction* of *Spilanthes filicaulis*
ALT: Alanine aminotransferase
AST: aspartate aminotransferase
GGT: Gamma-Glutamyltransferase
LDH: Lactate Dehydrogenase
AChE: Acetylcholine esterase

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