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# Evaluation of serum and tissue biochemical assays of *Rattus norvegicus* after sub-acute oral administration of ethyl acetate sub-fraction of *Spilanthes filicaulis*

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## Abstract

**Background** Plants medicinal potential is well established, yet unregulated use can lead to health risks without proper toxicological assessments. This study aimed to evaluate the sub-acute toxicity of the ethyl acetate sub-fraction of *Spilanthes filicaulis* (ESSF) in *Rattus norvegicus*.

**Methods** Seventy-two male Wistar rats (148 ± 8.0 g) were randomly divided into groups. Group I (control) received 0.5 ml of distilled water, and Groups II–VI were administered ESSF orally at 62.5, 125, 250, 500, and 750 mg/kg body weight, respectively. Serum along with tissue (liver, kidney, and brain) biochemical assays were analysed after 14 and 28 days.

**Results** The results showed no significant differences ($p > 0.05$) in total protein, albumin, serum electrolytes, creatinine, urea, or total and conjugated bilirubin between treatment and control groups. However, minor elevations were noted in some biomarkers at specific doses after 14 and 28 days of treatment, though these remained within normal physiological ranges. A significant increase in serum glutamate dehydrogenase (GDH) was observed after 14 days in treated groups compared to controls ($p < 0.05$), but this were normalised by day 28, suggesting a reversible effect. Alkaline phosphatase (ALP), GDH, and creatinine kinase (CK) activities in tissues showed no significant differences between treated and control groups after 28 days of treatment, indicating no long-term tissue damage.

**Conclusions** These findings suggest that sub-acute oral administration of ESSF does not induce significant biochemical abnormalities. This implies that ESSF is relatively safe at the tested doses and supports its potential for therapeutic development. However, further long-term studies are necessary to establish its safety profile fully.

**Keywords** Ethyl acetate sub-fraction, *Spilanthes filicaulis*, Serum, Tissue, Biochemical assays, *Rattus norvegicus*

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## Background

Plants, the principal photosynthetic eukaryote, play a unique role as life on Earth depends on them. In essence, plants are the cornerstone of our survival and well-being, providing essential resources for nourishment through food, culinary enjoyment and flavour, remedies and medicine, protection and shelter, attire and fabric, etc. Their significance cannot be overstated, as they underpin our existence [1]. The medicinal potency of plants is rooted in their unique chemical constituents that exert distinct biological effects on the human body, thereby conferring therapeutic advantages [2, 3].

*Spilanthes filicaulis*, a medicinal plant, belongs to the small African creeping plant species in the genus Spilanthes of the Asteraceae family, commonly known as African Cress. The plant thrives in moist environments, commonly found in shaded places or along streams. The leaves taste pungent and unique, with flowers arranged in a dense, rounded cluster at the stem tips. Traditionally, whole plants are cooked as soup and a food additive, and as such, they possess quality nutritional advantages among communities of Southwestern Nigerians. Commonly found in tropical and subtropical regions, this plant has been used in various cultures to treat ailments such as toothaches, inflammation, and fever, primarily due to its rich bioactive compounds. Recent studies have revealed that Spilanthes species possess various pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and analgesic effects [4]. These attributes make *Spilanthes filicaulis* a promising candidate for further investigation in modern pharmacology. For instance, there are scientific reports on the plant in treating toothache [5], stomach-ache, snake bites, malaria [6] and an antidote for poison [7] (Fig. 1).

Among the bioactive compounds in *Spilanthes filicaulis*, alkamides and flavonoids have drawn particular attention due to their potent biological activities [8]. Ethyl acetate sub-fractions of plant extracts have been used to isolate and concentrate these phytochemicals, which may enhance the plant's therapeutic potential [4]. However,

**Fig. 1** Spilanthes filicaulis (Schumach. & Thonn) Asteraceae whole plant

despite the traditional uses and increasing interest in *Spilanthes filicaulis*, there is a need for scientific evaluation of its safety profile and biochemical effects, especially in preclinical models.

Toxicological assessments are critical in evaluating the safety of plant-derived compounds for potential therapeutic use. Serum biochemical indices, which reflect the functional status of major organs such as the liver, kidney, and heart, are commonly used to assess the safety of compounds following administration. Parameters such as liver enzymes (e.g. alanine aminotransferase (ALT), aspartate aminotransferase (AST)), kidney function markers (e.g. creatinine, urea), and lipid profiles (e.g. cholesterol, triglycerides) provide insights into potential toxic effects that might arise from long-term or high-dose administration of a substance [9]. The increasing preference for the use of plant and plant-based products over conventional drugs has led to their widespread and unregulated use, often without proper dosage control or awareness of potential risks [10]. As a result, there is mounting evidence that long-term intake of these plant-based products can disrupt the body's natural balance, potentially causing harm to health. If left unmonitored, herbal products may lead to liver and kidney damage, genetic damage, inflammatory response, metabolic disorders, etc. [11].

The Wistar rat (*Rattus norvegicus*) is a widely used experimental animal model in toxicological studies due to its well-characterised physiology and ease of handling [12]. Sub-acute toxicity studies, which involve repeated administration of a test compound over a short period, offer insights into the potential adverse effects that could arise from chronic exposure. These studies are essential for identifying safe dosage ranges and understanding the systemic impact of bioactive compounds.

Scientific literature reports that the leaves of *Spilanthes filicaulis* have medicinal uses, including antimalarial, antioxidant, anti-inflammatory, and anti-venom [13]. However, there is a paucity of scientific reports on its toxicological properties. This study aims to evaluate the effects of the ethyl acetate sub-fraction of *Spilanthes filicaulis* on serum biochemical indices in male Wistar rats following sub-acute oral administration. Specifically, we seek to determine whether this sub-fraction adversely affects liver and kidney function, lipid metabolism, and overall systemic health. By conducting this research, we aim to provide valuable data on the safety profile of *Spilanthes filicaulis* and its potential for future use in therapeutic formulations.

The findings from this study will contribute to the growing body of knowledge on the safety and efficacy of traditional medicinal plants and provide a foundation for further research into the potential clinical applications of *Spilanthes filicaulis*. Moreover, the results may guide future investigations into the molecular mechanisms underlying the pharmacological effects of this plant, with a view to its potential development into safe and effective therapeutic agents.

## Methods

### Collection and authentication of plant

Whole plants of *S. filicaulis* were harvested from a garden in Igbara Odo-Ekiti, Ekiti State, Nigeria. The plant material was subsequently identified and authenticated by experts at the Herbarium of Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria.

### Preparation of ESSF extract

Fresh parts of *S. filicaulis* were air-dried for seven weeks, pulverised into powder and extracted with 80% methanol using Soxhlet extraction. The extract was evaporated to obtain a crude methanolic extract using Buchi Rotavapor R-210, which was later fractionated using solvent–solvent partitioning to yield hexane, ethyl acetate and butanol fractions [4]. Column chromatography was performed on the ethyl acetate fraction, and similar eluates were pooled into three sub-fractions (SFA, SFB, and SFC) using thin-layer chromatography. The sub-fractions were stored in sealed amber glass jars in the refrigerator pending further biological assays.

### Animals and experimental design

Seventy-two albino rats (*Rattus norvegicus*) of average weight 148 ± 8.0 g were grouped into six of twelve rats per group. They were acclimatised for seven days and administered treatment of sub-fraction C (SFC) extract of *Spilanthes filicaulis* for 14 and 28 days. All animals in each group received 0.5 mL of the appropriate sample solution orally as follows:

Group 1: Control (0.5 ml distilled water).

Group 2: 62.50 mg/kg body weight of the most active sub-fraction C extract of *S. filicaulis.*

Group 3: 125.0 mg/kg body weight of the most active sub-fraction C extract of *S. filicaulis.*

Group 4: 250.0 mg/kg body weight of the most active sub-fraction C extract of *S. filicaulis.*

Group 5: 500.0 mg/kg body weight of the most active sub-fraction C extract of *S. filicaulis.*

Group 6:750.0 mg/kg body weight of the most active sub-fraction C extract of *S. filicaulis.*

### Sample collection and preparation

Twelve hours after dosing on day fourteen, half of the rats in all groups were anaesthetised by chloroform inhalation in an air-tight chamber. Jugular venous blood was collected into plain sample bottles for biochemical analyses.

Blood samples in plain bottles were spun at 3,000 rpm for 10 min, and the supernatant serum was carefully pipetted into another properly labelled plain bottle. These were stored frozen until needed for analysis. Each rat's organs (kidneys, brain and liver) were harvested, cleansed of superficial connective blood/tissues in saline, weighed, homogenised in ice-cold 0.25 M sucrose solution (1:5 w/v), and labelled appropriately in a sample bottle. Homogenates were also stored overnight at −20 °C to ensure maximum release of enzymes. They were thawed and centrifuged at 10,000 rpm for 10 min in a refrigerated centrifuge (Thermo Scientific Sorvall Legend XTR), and the supernatants were pipetted into new plain tubes. These were stored at −20 °C for biochemical analyses. The remaining rats in all the groups were sacrificed after day 28, and the same procedure was thoroughly followed.

### Biochemical analysis

The serum total protein was determined using the biuret method described by Gornall et al. [12]. Serum albumin was determined using the technique of Doumas et al. [13]. Serum total bilirubin and conjugated bilirubin concentration were determined by adopting the method described by Jendrassik and Grof [14]. The serum urea and uric acid levels were determined by the methods of Weatherburn [15] and Fossati et al. [16], respectively. Serum creatinine concentration was estimated using Bartels and Bohmer's method [17].

#### *Total protein concentration*

Biuret reagent (1000 µL) was added to 250 µL of standard (bovine serum albumin) or diluted sample, mixed, and incubated in an incubator at room temperature for 30 min. Absorbance was then measure using spectrophotometer (Thermo Scientific Evolution 201) at 546 nm against a reagent blank [14].

#### *Serum albumin concentration*

Test tubes for blank, standard, and sample were prepared with 1000 µL working reagent. Then, 10 µL of distilled water, standard albumin solution, or sample were added, mixed, and incubated at room temperature for 5 min. Absorbance was measured using Spectrophotometer (Thermo Scientific Evolution 201) at 630 nm against the reagent blank [15].

#### *Serum total bilirubin concentration*

Blank and sample test tubes received 200 µL reagent 1 (sulphanilic acid). Reagent 2 (sodium nitrite, 50 µL) was added only to sample tubes. Then, 1000 µL reagent 3 (caffeine and sodium benzoate) and 200 µL diluted serum were added to both. After 10 min, 1000 µL reagent 4 (sodium tartarate and sodium hydroxide) was added, mixed, and incubated for 20 min. Absorbance was read at 578 nm against the sample blank.

#### *Conjugated bilirubin concentration*

A mixture of 200 µL reagent 1 (sulphanilic acid), 50 µL reagent 2 (sodium nitrite), 2000 µL 0.9% NaCl, and 200 µL sample was incubated at room temperature for 10min. Absorbance was read at 546 nm against a sample blank prepared without reagent 2 [16].

#### *Serum urea concentration*

100 µL working reagent (sodium nitroprusside and urease) was added to blank, standard, and sample test tubes. Distilled water, standard urea solution, or sample were then added, mixed, and incubated at 37 °C for 10 min. After adding phenol and sodium hypochlorite, the mixture was incubated for 15 min. Absorbance was read at 546 nm against the reagent blank [17].

#### *Serum uric concentration*

20 µL of standard uric acid solution or sample were mixed with 1000 µL pre-mixed working reagent (Hepes buffer, 3,5-Dichloro-2-hydroxybenzensulfonic, 4-aminophenazone, peroxidase, and uricase). After 15 min at room temperature, absorbance was read at 520 nm against a reagent blank [18].

#### *Serum creatinine concentration*

An aliquot (1,000 µL) containing working reagent (35 mmol/L of picric acid and 0.32 mmol/L NaoH, 1:1 v/v) was measured using a pipette into test tubes for standard and sample, respectively. Thereafter, 100 µL of standard creatinine solution (181 µmol/L creatinine) and sample was added into their respective test tubes, mixed and incubated at room temperature for 30 s. The absorbance was read at 492 nm [19].

#### *Alkaline phosphatase (ALP) activity*

The reaction mixture as described by Wright et al. [20] was initiated by adding 20 µL diluted sample with 1000 µL reagent, then measure absorbance using spectrophotometer (Thermo Scientific Evolution 201) at 405 nm at 0,1,2, and 3 min to calculate the change in absorbance per minute.

#### *Glutamate dehydrogenase (GDH) activity*

The reaction mixture as described by Shimizu et al. [21] consists of Tris–HCl buffer, α-Ketoglutarate, $NH_4Cl$, NADH, and EDTA. A 50 µL diluted sample was added, and the reaction was incubated using an incubator at room temperature for 5 min. The decrease in absorbance at 340 nm per minute was measured against a reagent blank.

#### *Creatine kinase (CK) activity*

The reaction mixture as described by Di Witt and Trendelenburg [22] was initiated by combining 200 µL of reagent1 and 50 µL of reagent 2. A 10 µL diluted sample was added, mixed, and incubated at room temperature for 5 min. Absorbance was measured at 340 nm using spectrophotometer (Thermo Scientific Evolution 201) against distilled water at 0, 1, 2, and 3 min.

### Statistical analysis

The one-way analysis of variance (ANOVA), followed by Duncan's post hoc multiple comparisons, was used to analyse and compare the results at a 95% confidence level. A *p*-value less than 0.05 were considered significant. The results were presented as mean ± standard error of the mean (SEM). Graph of the data obtained was performed using GraphPad Prism Software (GraphPad Software, Inc., USA).

## Results

### Serum electrolytes

The sub-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 ($p > 0.05$) alter the serum concentrations of $Na^+$, $Ca^{2+}$, $K^+$, $Cl^-$, $PO_4^{3-}$ and $HCO_3^-$ on day 14 and day 28 compared to control groups (Tables 1, 2).

### Serum biomolecules

The sub-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 ($p > 0.05$) alter the serum concentrations of creatinine, urea and uric acid on day 14 and day 28 compared to control groups (Tables 3, 4).

The sub-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 ($p > 0.05$) alter the serum total protein, albumin, and globulin compared to the control. A similar trend was observed for conjugated bilirubin and total bilirubin activity in the serum on day 14 and day 28 compared to the control (Tables 5, 6).

### Alkaline phosphatase (ALP) activity

The sub-fraction C of *Spilanthes filicaulis* of ethyl acetate partition extract at all doses investigated on the rats after 28 days of oral administration did not significantly ($p > 0.05$) affect the activities of ALP in the serum, brain, kidneys, and liver on day 14 and day 28 compared to the control (Figs. 2, 3).

### Glutamate dehydrogenase (GDH) activity

The sub-fraction C of *Spilanthes filicaulis* ethyl acetate partition extract at most doses investigated on the rats after 28 days of oral administration did not have a

**Table 1** Effects of oral administration of sub-fraction C (SFC) from ethyl acetate partitioned extract of *Spilanthes filicaulis* on serum electrolyte concentrations of rats on day 14

| Treatment | $Na^+$ (mmol/L) | $Ca^{2+}$ (mmol/L) | $K^+$ (mmol/L) | $Cl^-$ (mmol/L) | $PO_4^{3-}$ (mmol/L) | $HCO_3^-$ (mmol/L) |
|---|---|---|---|---|---|---|
| Control | $140.26 \pm 1.20^a$ | $4.25 \pm 0.13^b$ | $4.31 \pm 0.21^c$ | $110.01 \pm 1.10^d$ | $7.23 \pm 0.13^e$ | $28.02 \pm 0.68^f$ |
| 62.50 mg/kg body weight | $141.09 \pm 1.17^a$ | $4.01 \pm 0.16^b$ | $4.24 \pm 0.15^c$ | $108.87 \pm 1.18^d$ | $7.18 \pm 0.10^e$ | $26.80 \pm 0.90^f$ |
| 125 mg/kg body weight | $139.88 \pm 1.31^a$ | $4.32 \pm 0.10^b$ | $4.21 \pm 0.12^c$ | $110.20 \pm 1.07^d$ | $7.41 \pm 0.12^e$ | $27.06 \pm 0.84^f$ |
| 250 mg/kg body weight | $140.71 \pm 1.27^a$ | $4.24 \pm 0.20^b$ | $4.27 \pm 0.11^c$ | $110.08 \pm 1.16^d$ | $7.20 \pm 0.17^e$ | $25.97 \pm 0.81^f$ |
| 500 mg/kg body weight | $140.69 \pm 1.12^a$ | $4.27 \pm 0.14^b$ | $4.19 \pm 0.18^c$ | $109.90 \pm 1.00^d$ | $7.22 \pm 0.15^e$ | $28.10 \pm 0.77^f$ |
| 750 mg/kg body weight | $142.01 \pm 1.09^a$ | $4.20 \pm 0.15^b$ | $4.22 \pm 0.24^c$ | $110.06 \pm 1.07^d$ | $7.25 \pm 0.10^e$ | $26.92 \pm 0.80^f$ |

Data are presented as means ± SEM of six replicates. Means with different superscripts within a column are significantly different at $p < 0.05$

**Table 2** Effects of oral administration of sub-fraction C (SFC) from ethyl acetate partitioned extract of *Spilanthes filicaulis* on serum electrolyte concentrations of rats on day 28

| Treatment | $Na^+$ (mmol/L) | $Ca^{2+}$ (mmol/L) | $K^+$ (mmol/L) | $Cl^-$ (mmol/L) | $PO_4^{3-}$ (mmol/L) | $HCO_3^-$ (mmol/L) |
|---|---|---|---|---|---|---|
| Control | $141.44 \pm 1.20^a$ | $3.75 \pm 0.13^b$ | $4.80 \pm 0.28^c$ | $104.66 \pm 1.24^d$ | $6.83 \pm 0.10^e$ | $28.60 \pm 0.80^f$ |
| 62.50 mg/kg body weight | $140.88 \pm 1.17^a$ | $3.78 \pm 0.16^b$ | $4.72 \pm 0.25^c$ | $105.03 \pm 1.38^d$ | $6.88 \pm 0.12^e$ | $27.90 \pm 0.78^f$ |
| 125 mg/kg body weight | $139.88 \pm 1.31^a$ | $3.90 \pm 0.10^b$ | $4.69 \pm 0.30^c$ | $105.20 \pm 1.55^d$ | $6.91 \pm 0.15^e$ | $27.88 \pm 0.85^f$ |
| 250 mg/kg body weight | $141.60 \pm 1.27^a$ | $3.81 \pm 0.20^b$ | $4.77 \pm 0.41^c$ | $104.71 \pm 1.46^d$ | $7.01 \pm 0.14^e$ | $27.77 \pm 0.69^f$ |
| 500 mg/kg body weight | $140.25 \pm 1.12^a$ | $4.01 \pm 0.14^b$ | $4.80 \pm 0.28^c$ | $106.00 \pm 1.60^d$ | $6.92 \pm 0.15^e$ | $29.06 \pm 0.83^f$ |
| 750 mg/kg body weight | $141.10 \pm 1.09^a$ | $3.82 \pm 0.15^b$ | $4.74 \pm 0.26^c$ | $104.85 \pm 1.62^d$ | $6.85 \pm 0.18^e$ | $28.79 \pm 0.87^f$ |

Data are presented as means ± SEM of six replicates. Means with different superscripts within a column are significantly different at $p < 0.05$

**Table 3** Effects of oral administration of sub-fraction C (SFC) from ethyl acetate partitioned extract of *Spilanthes filicaulis* on selected kidney function indices of rats on day 14

| Treatment | Creatinine (µmol/L) | Urea (mg/dL) | Uric Acid (mg/dL) |
|---|---|---|---|
| Control | $62.66 \pm 0.62^{a}$ | $20.09 \pm 0.30^{b}$ | $1.40 \pm 0.20^{c}$ |
| 62.50 mg/kg body weight | $61.68 \pm 0.80^{a}$ | $21.15 \pm 0.27^{b}$ | $1.35 \pm 0.31^{c}$ |
| 125 mg/kg body weight | $63.09 \pm 0.46^{a}$ | $20.27 \pm 0.19^{b}$ | $1.36 \pm 0.17^{c}$ |
| 250 mg/kg body weight | $61.40 \pm 0.55^{a}$ | $20.11 \pm 0.37^{b}$ | $1.39 \pm 0.28^{c}$ |
| 500 mg/kg body weight | $62.51 \pm 0.77^{a}$ | $21.03 \pm 0.40^{b}$ | $1.47 \pm 0.20^{c}$ |
| 750 mg/kg body weight | $61.97 \pm 0.81^{a}$ | $20.13 \pm 0.20^{b}$ | $1.41 \pm 0.10^{c}$ |

Data are presented as means ± SEM of six replicates. Means with different superscripts within a column are significantly different at $p < 0.05$.

**Table 4** Effects of oral administration of sub-fraction C (SFC) from ethyl acetate partitioned extract of *Spilanthes filicaulis* on selected kidney function indices of rats on day 28

| Treatment | Creatinine (µmol/L) | Urea (mg/dL) | Uric Acid (mg/dL) |
|---|---|---|---|
| Control | $61.13 \pm 0.55^{a}$ | $21.02 \pm 0.18^{b}$ | $1.37 \pm 0.23^{c}$ |
| 62.50 mg/kg body weight | $62.07 \pm 0.63^{a}$ | $22.20 \pm 0.25^{b}$ | $1.38 \pm 0.27^{c}$ |
| 125 mg/kg body weight | $62.84 \pm 0.65^{a}$ | $20.87 \pm 0.22^{b}$ | $1.38 \pm 0.18^{c}$ |
| 250 mg/kg body weight | $62.50 \pm 0.51^{a}$ | $20.95 \pm 0.21^{b}$ | $1.36 \pm 0.20^{c}$ |
| 500 mg/kg body weight | $62.44 \pm 0.48^{a}$ | $21.78 \pm 0.20^{b}$ | $1.40 \pm 0.17^{c}$ |
| 750 mg/kg body weight | $62.17 \pm 0.70^{a}$ | $21.55 \pm 0.19^{b}$ | $1.42 \pm 0.26^{c}$ |

Data are presented as means ± SEM of six replicates. Means with different superscripts within a column are significantly different at $p < 0.05$

**Table 5** Effects of oral administration of sub-fraction C (SFC) from ethyl acetate partitioned extract of *Spilanthes filicaulis* on selected liver function indices of rats on day 14

| Treatment | Total Protein (g/L) | Albumin (g/L) | Globulin (g/L) | Total Bilirubin (µmol/L) | Conjugated Bilirubin (µmol/L) |
|---|---|---|---|---|---|
| Control | $30.76 \pm 0.51^{a}$ | $19.20 \pm 0.12^{b}$ | $11.56 \pm 0.11^{c}$ | $26.90 \pm 0.10^{d}$ | $9.64 \pm 0.12^{e}$ |
| 62.50 mg/kg body weight | $29.80 \pm 0.37^{a}$ | $18.80 \pm 0.18^{b}$ | $11.00 \pm 0.17^{c}$ | $28.02 \pm 0.10^{d}$ | $10.08 \pm 0.17^{e}$ |
| 125 mg/kg body weight | $29.46 \pm 0.63^{a}$ | $19.69 \pm 0.23^{b}$ | $9.77 \pm 0.13^{c}$ | $28.47 \pm 0.11^{d}$ | $9.30 \pm 0.10^{e}$ |
| 250 mg/kg body weight | $30.29 \pm 0.50^{a}$ | $18.91 \pm 0.22^{b}$ | $11.38 \pm 0.19^{c}$ | $27.91 \pm 0.10^{d}$ | $9.46 \pm 0.09^{e}$ |
| 500 mg/kg body weight | $31.00 \pm 0.71^{a}$ | $20.02 \pm 0.18^{b}$ | $10.98 \pm 0.11^{c}$ | $27.02 \pm 0.13^{d}$ | $9.71 \pm 0.10^{e}$ |
| 750 mg/kg body weight | $30.30 \pm 0.60^{a}$ | $19.13 \pm 0.16^{b}$ | $11.17 \pm 0.10^{c}$ | $28.10 \pm 0.12^{d}$ | $10.00 \pm 0.12^{e}$ |

Data are presented as means ± SEM of six replicates. Means with different superscripts within a column are significantly different at $p < 0.05$

**Table 6** Effects of oral administration of sub-fraction C (SFC) from ethyl acetate partitioned extract of *Spilanthes filicaulis* on selected liver function indices of rats on day 28

| Treatment | Total Protein (g/L) | Albumin (g/L) | Globulin (g/L) | Total Bilirubin (µmol/L) | Conjugated Bilirubin (µmol/L) |
|---|---|---|---|---|---|
| Control | $31.03 \pm 0.46^{a}$ | $20.06 \pm 0.13^{b}$ | $10.97 \pm 0.16^{c}$ | $27.20 \pm 0.10^{d}$ | $10.55 \pm 0.12^{e}$ |
| 62.50 mg/kg body weight | $29.33 \pm 0.70^{a}$ | $20.00 \pm 0.18^{b}$ | $9.33 \pm 0.14^{c}$ | $29.00 \pm 0.10^{d}$ | $10.98 \pm 0.17^{e}$ |
| 125 mg/kg body weight | $32.00 \pm 0.65^{a}$ | $19.88 \pm 0.20^{b}$ | $12.12 \pm 0.18^{c}$ | $27.77 \pm 0.11^{d}$ | $10.70 \pm 0.10^{e}$ |
| 250 mg/kg body weight | $29.97 \pm 0.53^{a}$ | $19.97 \pm 0.20^{b}$ | $10.00 \pm 0.13^{c}$ | $28.21 \pm 0.10^{d}$ | $11.06 \pm 0.09^{e}$ |
| 500 mg/kg body weight | $31.81 \pm 0.50^{a}$ | $20.80 \pm 0.15^{b}$ | $11.01 \pm 0.20^{c}$ | $28.00 \pm 0.13^{d}$ | $10.41 \pm 0.10^{e}$ |
| 750 mg/kg body weight | $31.10 \pm 0.80^{a}$ | $20.22 \pm 0.18^{b}$ | $10.88 \pm 0.16^{c}$ | $28.80 \pm 0.12^{d}$ | $10.90 \pm 0.12^{e}$ |

Data are presented as means ± SEM of six replicates. Means with different superscripts within a column are significantly different at $p < 0.05$

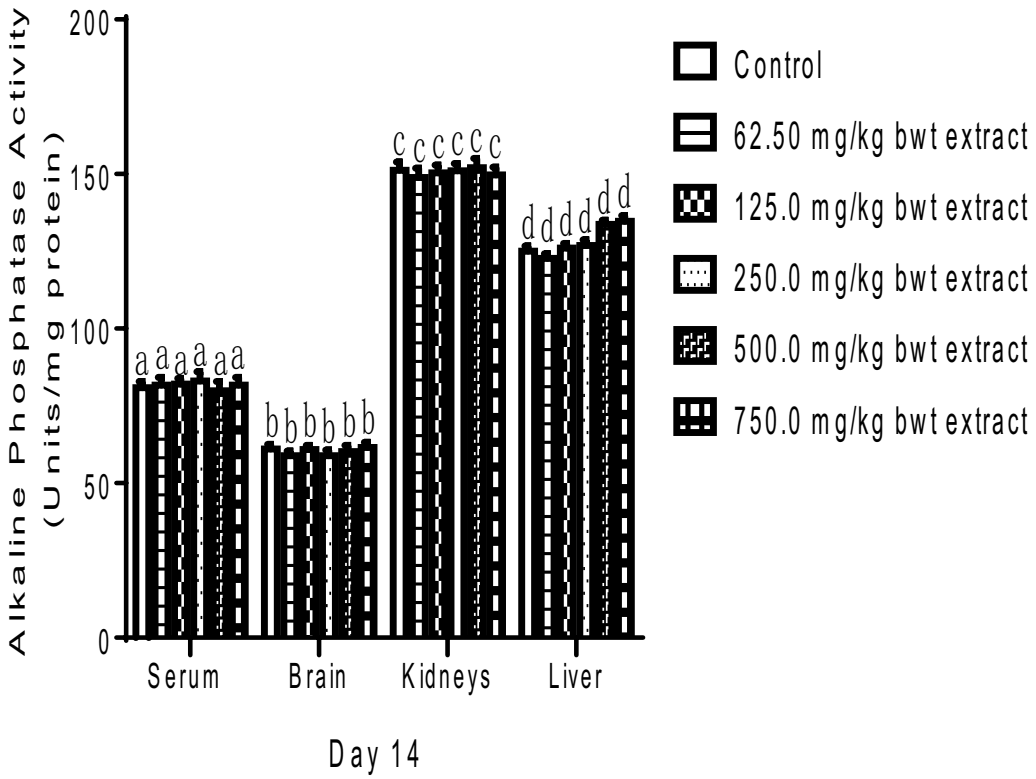


**Fig. 2** Effects of oral administration of sub-fraction C (SFC) from ethyl acetate partitioned extract of *Spilanthes filicaulis* on specific activity of alkaline phosphatase (ALP) in serum, brain, kidneys and liver of rats on day 14. Values are means ± SEM of six replicates. Bars with similar alphabets are not significantly different ($p > 0.05$). bwt = body weight

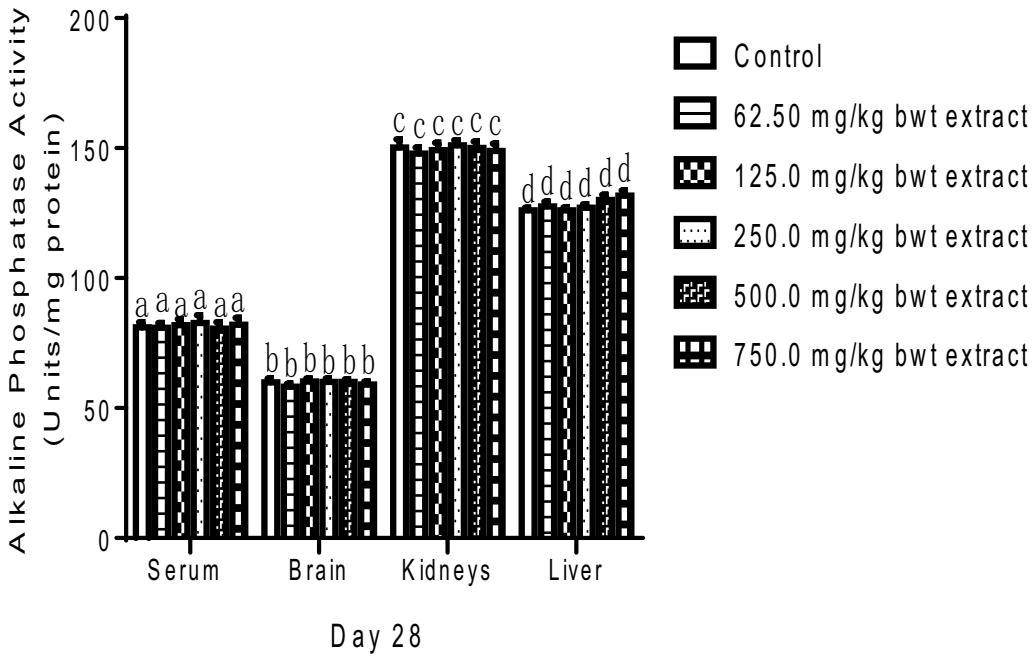


**Fig. 3** Effects of oral administration of sub-fraction C (SFC) from Ethyl acetate partitioned extract of *Spilanthes filicaulis* on specific activity of alkaline phosphatase (ALP) in serum, brain, kidneys and liver of rats on day 28. Values are means ± SEM of six replicates. Bars with similar alphabets are not significantly different ($p > 0.05$). bwt = body weight

significant effect ($p > 0.05$) on the activities of GDH in the serum and liver on day 14 and day 28 compared to control. However, there was a significant increase ($p < 0.05$) in GDH activities in the liver at the highest dose of 750 mg/kg when compared to other doses, and this was the control group on day 14; this increase was later offset after 28 days of oral administration of the extract (Fig. 4).

#### Creatinine kinase (CK) activity

The sub-fraction C of *Spilanthes filicaulis* ethyl acetate partition extract at most doses investigated on the rats after 28 days of oral administration did not have a significant effect ($p > 0.05$) on the activities of CK in the serum and brain on day 14 and day 28 compared to control. However, there was a significant increase ($p < 0.05$) in CK activities in the brain at the higher doses of 500 and 750 mg/kg when compared to other doses and the control group on day 14; this increase was later offset after 28-day oral administration of the extract (Fig. 5).

## Discussion

*Spilanthes filicaulis* have been used to treat a variety of conditions traditionally. However, to the best of our knowledge, there is a shortage of information and literature reports concerning the safety and toxicity of this plant. This study thus evaluated the sub-acute toxicological effects of ESSF in male Wistar rats. Medicinal plants show increasing scientific evidence of their therapeutic benefits [12]. However, research has also shown that some of these plants can harm the body when used on long-term [23]. Repeated oral administration of plant extracts can potentially harm vital organs (liver and kidney), crucial in metabolising foreign substances. Consequently, monitoring biochemical indices is essential to gauge the effects on these organs and ensure their functional integrity.

The liver is a vital organ in animals receiving the highest blood supply, accounting for 25% of the total cardiac output [24]. The liver being the major site of drug metabolism encounters many foreign substances entering the body [25]. Since the liver performs diverse functions, no single test can thoroughly estimate its functionality [26]. Liver function biomarkers evaluated in this study are useful screening tools that effectively detect hepatic dysfunction, such as total serum protein, albumin, globulin, bilirubin, alkaline phosphatase (ALP), and glutamate dehydrogenase (GDH). The damage to the liver cells results in tissue content leakage into the bloodstream [27].

The serum's total protein content comprises albumin (60%) and globulin (40%). Albumin produced in the liver is the main constituent of serum total protein responsible

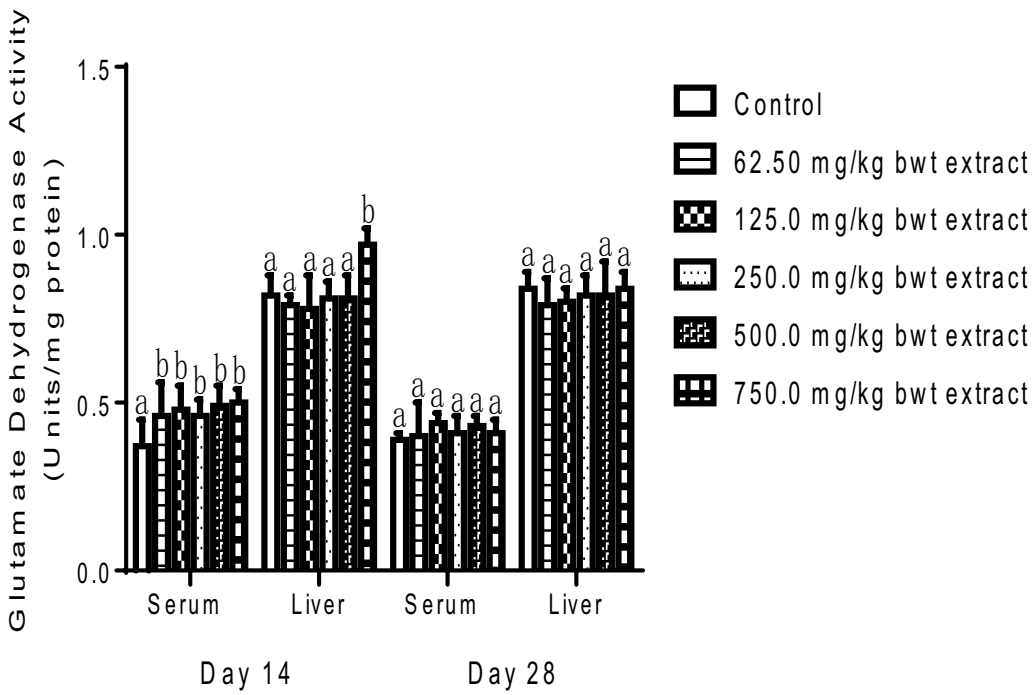


**Fig. 4** Effects of oral administration of sub-fraction C (SFC) from ethyl acetate partitioned extract of *Spilanthes filicaulis* on specific activity of glutamate dehydrogenase (GDH) in serum and liver of rats on day 14 and 28. Values are means ± SEM of six replicates. Bars with similar alphabets are not significantly different ($p > 0.05$). bwt = body weight

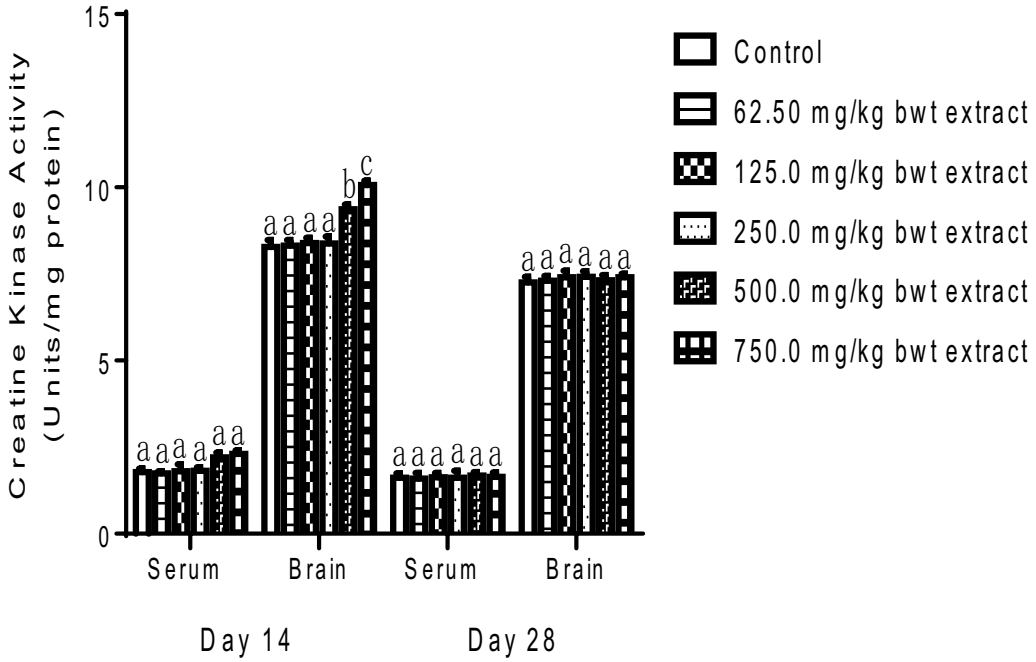


**Fig. 5** Effects of oral administration of sub-fraction C (SFC) from ethyl acetate partitioned extract of *Spilanthes filicaulis* on specific activity of creatine kinase (CK) in serum and brain of rats on day 14 and 28. Values are means ± SEM of six replicates. Bars with similar alphabets are not significantly different ($p > 0.05$). bwt = body weight

for maintaining plasma osmotic pressure and movement of lipids and hormones [28, 29]. Albumin activities are decreased in liver diseases such as cirrhosis [30] and malaria [6]. Serum total protein, albumin, and globulin activities indicate liver function since they are produced by the liver [31]. Globulins mainly perform immunological functions. Serum total protein, albumin and globulin concentrations at all doses, after 28 days of oral administration of SFC on rats, were significantly not altered on day 14 and day 28 compared to the control. This may suggest that the extract does not seriously affect hepatocyte function in the rats or induce any cytotoxic severe damage to the liver at the tested doses (Tables 5, 6). These outcomes are in line with report by Umar et al., [32] on sub-chronic administration of *Halea ciliata* on rats.

Bilirubin, formerly known as haematoidin, is the yellowish breakdown product of normal heme. The importance of the liver in bilirubin metabolism cannot be overemphasised. Thus, any disease condition that affects liver integrity can directly interfere with the liver handling of bilirubin and its attendant complications [33]. Bilirubin is a vital metabolic product of the blood with diagnostic and biological values [34]. Bilirubin bound non-covalently to serum albumin, increasing solubility [35]. It is then selectively taken up by the liver, biotransformed upon conjugation with glucuronic acid in the endoplasmic reticulum by a transferase, and secreted into bile [36]. The high amount of total bilirubin in blood causes jaundice, implying problems in liver metabolic function, which could result from reduced hepatocyte uptake, impaired bilirubin conjugation, or reduced bilirubin secretion [37]. Serum total and conjugated bilirubin activities, at all doses after 28 days of oral administration of SFC on rats, compared favourably with control on day 14 and day 28. This may suggest that the sample may not have caused uncontrollable heme degradation, which might have resulted in the accumulation of bilirubin within the circulatory system, and that the conjugation functions of the liver were conserved (Tables 5, 6). Our finding agrees with earlier report by Chung et al., [38] who observed no significant alteration in the serum bilirubin levels after oral administration of *Momordica charantia* extract, and Cidjeu et al. [39].

Alkaline phosphatase (ALP) is a membrane-bound hydrolase enzyme responsible for dephosphorylating many types of molecules. Although present in all tissues throughout the body, ALP is mainly concentrated in the liver, kidney, bile duct, bone, and placenta [40]. Enzyme activities of ALP in the serum and liver, at all doses after 28 days of oral administration of SFC on rats, were not significantly altered on day 14 and day 28 compared to control. This may suggest that there was no liver damage arising from the 28-day oral administration of the sample, which may not have obstructed the hepatobiliary

duct in the hepatocytes, thus not resulting in leakage of the enzymes into the circulatory system (Fig. 2, 3).

Glutamate dehydrogenase (GDH) is a useful biochemical indicator of injury to the mitochondria membrane [41], catalysing the removal of the amino group of glutamate as $NH_4^+$ during oxidative deamination reaction. This enzyme also catalyses the reductive amination of α-ketoglutarate forming glutamate [42], which depends on high-energy activity, while low-energy activity prefers the oxidative deamination reaction to form α-ketoglutarate [43]. The ubiquitous enzyme represents a critical link between catabolic and anabolic pathways in eukaryotes. The activity of this enzyme can serve to equip the tricarboxylic acid cycle with α-keto acids and to take off ammonia from circulation for the synthesis of urea. The result of the oral administration of SFC on GDH enzyme activities in the serum on day 14 was slightly higher across the treatment groups compared to the control. At the same time, the increase was only noticed in the liver at the high dose compared to other treatments and controls. However, this effect in the serum and liver was modulated on day 28, which was not significantly altered compared to control (Fig. 4). This may suggest that the sample did not outcome mitochondrial damage in the rat's liver.

ALP activities in the brain were not significantly altered at all doses on day 14 and day 28, compared favourably to the control. This suggests that the sample treatments may not adversely affect energy homeostasis in the brain cells. CK is the gold-standard diagnostic serum marker for muscle damage, and its activity is elevated in any condition that causes muscle injury. Oral administration of SFC on CK was slightly increased, which was significant at the high dose of the sample when compared to other treatment groups and the control in the brain on day 14. However, on day 28, these effects were modulated so that the CK activities in the serum and brain, at all doses, were not significantly altered compared to the control (Fig. 5).

The kidney is particularly endangered to toxicants due to its high blood flow and filtration function, which exposes it to a broad volume of toxins that may accumulate and concentrate in the renal tubules [44]. Toxic substances such as drugs, heavy metals, chemicals, and immunological complexes can afflict injury to the kidney and, in return, incapacitate it from performing prominent excretory functions that may lead to renal failure [45, 46]. Creatinine and urea are non-protein metabolites that are removed by the body next to glomerular filtration, thus assessing the activity of creatinine, serum urea, and electrolytes ($Na^+$, $K^+$, $Cl^-$, $HCO_3^-$) are extremely important and sensitive biochemical markers which can be used to evaluate renal function [47, 48].

Creatinine is produced endogenously in the muscles by non-enzymic action on creatinine phosphate [49]. Serum creatinine levels are directly proportional to the body's muscle mass, and its concentration remains relatively constant due to consistent production. The kidney readily excretes it. When creatinine and urea are reserved in the blood, it suggests an impairment of the functionality of the kidney [50]. Elevation in creatinine, urea and uric acid in serum activities may indicate renal dysfunction [51, 52]. Urea is a waste product resulting from protein metabolism [53]. Urea disseminates in the bloodstream until it is removed by the kidneys through filtration and subsequently excreted in the urine [54]. Serum urea, uric acid and creatinine concentrations at all doses after 28 days of oral administration of SFC on rats compared favourably with control on day 14 and day 28 (Tables 3, 4). This may suggest that the sample may not cause systemic toxicity that could have led to elevated activity in the serum of these non-protein metabolites, which could have emanated from its reduced excretion. Also, the urea cycle may not have been affected, and the glomerular filtration by the kidney was not compromised.

Extracellular and intracellular compartments are made up of inorganic electrolytes, which in their dissociated forms help to improve the transport of water and electrolytes between the body fluid compartments [55]. Abnormal activity of serum/plasma electrolytes could easily indicate that kidney function has been impaired [56], as electrolyte balance could show the possibility of proper maintenance of homeostasis. Serum $Na^+$, $Ca^{2+}$, $K^+$, $Cl^-$, $PO_4^{3-}$ and $HCO_3^-$concentrations at all doses after 28 days of oral administration of SFC on rats were not significantly altered in all groups and compared favourably with control on day 14 and day 28 (Tables 1, 2). This suggests that the sample may not adversely interfere with serum electrolyte balance, suggesting a possible good osmoregulatory function of the kidney. In general, the result of our findings do not agree with the report by Orisakwe et al. [57] and Abubakar et al. [45] but are in concert with studies of Bushra Abbas [58], Umar et al. [59], Yusuf et al. [60], and Jegnie et al. [61] who reported no significant difference ($p < 0.05$) in the urea, uric acid, creatinine, electrolytes activity of the treated rats when compared with the control.

Assessing kidney tissue damage can be achieved by measuring the serum activity of ALP, a membrane-bound enzyme. When tissue damage occurs, these enzymes are released from the damaged cells into the bloodstream, leading to elevated serum levels of ALP, indicating renal injury. ALP activities in the kidneys and serum at all doses after 28 days of oral administration of SFC on rats compared favourably with control

on day 14 and day 28. This finding may suggest that the sample did not induce a distortion of the kidney plasma membrane.

## Conclusion

The stability of serum electrolytes, biomolecules, and some of the cellular enzymes assayed suggests no disruption of the rat's essential physiological processes. Therefore, we certify that the SFC extract is non-toxic and safe. However, further experimentation needs to be done about isolation and characterisation to explore active constituents and assess the toxicity of the sub-fraction in combination with other extracts/compounds.

### Abbreviations

| | |
|---|---|
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| CK | Creatinine kinase |
| ESSF | Ethyl acetate sub-fraction of *Spilanthes filicaulis* |
| GDH | Glutamate dehydrogenase |
| SEM | Standard error of the mean |
| SFA | Sub-fraction A |
| SFB | Sub-fraction B |
| SFC | Sub-fraction C |

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### Declaration of Compliance with Plant-Related Regulations

Plant species studied: *Spilanthes filicaulis.* Study location: Igbara Odo-Ekiti, Ekiti South West, Ekiti State 7.5038°N, 5.0625°E. The study plant was identified and authenticated at IFE Herbarium, Obafemi Awolowo University, Ile-Ife with voucher specimen number IFE/17571.

### Author contributions

All authors contributed to this study. Conceptualisation, material preparation and investigation, data collection, and analysis were performed by BEO, OBO, and AAA. MGA prepares the serum and carefully harvested the tissues for biochemical assay. Supervision of the study was done by EAB. The first draft of the manuscript was written by BEO, and all authors reviewed its perfection to the final edition of the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

All data are available on request from corresponding author.

## Declarations

### Ethical approval and consent to participate

The study was ethically reviewed by the University of Ilorin, Ilorin Nigeria Ethical Committee and an approval number (UERC Approval Number: UERC/ASN/2023/2475) was issued.

### Consent for publication

The authors declare no conflict of interest.

### Competing interests

The authors declare that they have no competing interests.

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