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ORIGINAL RESEARCH ARTICLE

Acute and Sub-Acute Toxicity Studies of Aqueous Leaf Extract of *Launaea taraxacifolia* (Willd.) in Wistar Rats

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ABSTRACT

Aim: This study aimed to evaluate the acute and sub-acute toxicity profile of aqueous leaf extract of *Launaea taraxacifolia* in Wistar rats.

Methods: Acute toxicity was investigated using Lorke's method, while sub-acute toxicity was assessed using the Organisation for Economic Co-operation and Development guideline. The first phase of the acute toxicity study had nine Wistar rats randomly divided into three groups (n = 3) and administered doses of 10, 100 and 1000mg/kg (orally [*po*]) leaf extract of LT, respectively. In the second phase, four groups (n = 1) received doses of 1200, 1600, 2900 and 5000 mg/kg (*po*) of the extract, respectively. For the sub-acute toxicity study, 20 rats were randomly divided into four groups (n = 5). Group 1 received 10ml/kg distilled water, while Groups 2–4 received 250, 500 and 1000mg/kg (*po*) of the extract, respectively, for 28 days.

Results: Acute toxicity result showed no mortality in both phases of the study. In the sub-acute studies, significant increases in platelets in all treatment groups and RBC at the highest dose (1000 mg/kg) of the extract were observed ($P < 0.05$). There was a significant increase in sodium and potassium ions, while the liver function indices showed a significant elevation in alanine transaminase and aspartate transaminase at 500 and 1000mg/kg treatment doses. Histological examinations of the rat's kidney, liver, heart and spleen all showed normal cells; only follicular hyperplasia, congestion and oedema were seen in the spleen for groups treated with 500 and 1000mg/kg doses of the plant.

KEYWORDS:
ethnopharmacological; herbal vegetable; hepatotoxic; *Launaea taraxacifolia*; nephrotoxic; sub-acute toxicity study

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Conclusion: The aqueous leaf extract of *L. taraxacifolia* is relatively non-toxic. But can be hepatotoxic and nephrotoxic with long-term use of higher doses.

INTRODUCTION

The use and dependence on plants for food and medicinal purposes in developing economies such as in Africa and Asia are still high despite great strides achieved in orthodox medicines.[1] The World Health Organization (WHO) estimates that over 80% of the world's population, including the developed and developing countries, still rely on traditional medicines.[1–3] The use of herbal products for medicinal benefits still plays significant roles in many countries, including Nigeria.[4,5] Notwithstanding many objections and warnings by WHO and health authorities due to lack of scientific and systematic validation of such remedies, traditional herbal remedies are still widely consumed and accepted in many African countries and other parts of the world.[5,6] The food security in Africa is at a threat due to various political and calamities issues like drought due to global warming, increased cost of agricultural production, unstable pricing, internal/external conflicts and insecurities,[7,8] hence necessitating the need for cheaper viable alternatives for food and medicines in the continent.

Launaea taraxacifolia (Willd.) is an economically viable vegetable plant with numerous ethnopharmacological potentials. It is cultivated in tropical Africa where it serves as food, medicine and fodder of animals.[9] The plant has its origin in Ethiopian highlands and is a domesticated vegetable in Nigeria, Senegal and Benin.[8,10] *L. taraxacifolia* is commonly called wild lettuce and has local Nigerian names such as "efo yanrin" in Yoruba, "ugu" in Igbo and "nonon barya" in Hausa.[9,10] The plant is of high economic value due to its multiple uses. It is eaten as a vegetable because it is rich in proteins, essential fatty acids, vitamins, minerals and is highly fibrous. All of which significantly fill the nutrition deficiencies in the continent.[7,9–11] The plant is also a potential source of medicines with various pharmacological properties including, anti-inflammatory, anti-nausea antibiotic, anti-anaemic, anti-poison, anti-venom, cholesterol reduction, sedative, anti-diabetic and anti-cough.[8–10,12] Extract from the plant has also shown to have fungicidal and nematocidal properties.[9] The ethnobotanical, phytochemical and pharmacological potentials of *L. taraxacifolia* is previously evaluated et al.[8] Hence this study only aimed to investigate the acute and sub-acute toxicity of *L. taraxacifolia* (Willd.).

MATERIALS AND METHODS

PLANT COLLECTION AND EXTRACT PREPARATION

The leaf of *L. taraxacifolia* was collected from the medicinal plant garden of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, in February 2019. The plant was identified and authenticated in the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. A specimen with voucher number (PCG/UDUS/LLTF/0001) was deposited in the Departmental herbarium.

The study was carried out between February and September 2019. Extraction of *L. taraxacifolia* was performed according to a method already described by Alkali et al.[13] The freshly collected were dried under the shade for 14 days. Five hundred grams of the dried plant was pulverised to powder form and subjected to 72 hours of cold maceration with distilled water. After maceration, the mixture was filtered using Whatman filter paper (size 1.5), concentrated in the water bath and oven-dried at 40. This dried extract was transferred into an airtight container and for further analysis.

EXPERIMENTAL ANIMALS

Adult Wistar rats of both sexes (weighing 120–170g) obtained from the Animal care facility of Ahmadu Bello University Zaria were used for the study. The animals were housed for one month under standard laboratory conditions (12 hours light/dark cycle, in a room temperature, maintained at 24) in the experimental animal handling facility of the Faculty of Pharmaceutical Science, Usmanu Danfodiyo University Sokoto. They were fed with a commercially available rat pelleted diet (livestock feed) with *ad libitum* access to water throughout the experiment. The study protocols were approved by the Institutional Animal Care and Use Committee, Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto. PTAC/Lt/(Ae)OT/33-21

ACUTE TOXICITY STUDY

Oral acute toxicity was studied using the method described elsewhere.[14] This study was conducted in two phases. In the first phase, nine rats of both sexes fasted for 24 hours were randomly distributed into three groups with three rats each. Groups 1, 2 and 3 received 10, 100 and 1000mg/kg doses of the extract; were observed for 24 hours for any signs of toxicity and mortality. In the second phase, four rats were administered with 1200, 1600, 2900 and 5000mg/kg doses of the extract. The median lethal dose was determined by calculating the geometric mean of the highest dose that survived and the lowest dose that killed the animal.

SUB-ACUTE TOXICITY STUDY

The sub-acute toxicity profile was carried out using the Organisation for Economic Co-operation and Development 407 protocol.[13,15] Twenty previously fasted (24 hours) Wistar albino rats were divided into four groups of 5 animals each, and the

following treatments were carried out once a day for 28 days orally:

- Group1 (control): received 10 ml/kg/day of distilled water.
- Group 2: 250 mg/kg of the extract.
- Group 3: 500 mg/kg.
- Group 4: 1000 mg/kg.

The rats had *ad libitum* access to food and water throughout the experimental period; were observed daily for general symptoms of toxicity and mortality. The animals were euthanized on day 29 of the study. Before euthanasia, blood samples were collected by cardiac puncture for biochemical and haematological analysis. The heart, liver, kidney and spleen was excised and fixed with 10% formal saline for histopathological investigations.

HAEMATOLOGICAL INVESTIGATION

A portion of the collected blood stored in ethylenediaminetetraacetic acid tubes was used to carry out the following haematological analysis in the department of Haematology Usmanu Danfodiyo University Teaching Hospital (UDUTH): white blood cell count (WBC) and differentials, haematocrit (HCT), haemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume and platelet count.

BIOCHEMICAL INVESTIGATION

A portion of the blood sample previously collected and stored was subjected to the biochemical investigation such as liver function tests, determination of electrolytes, urea and creatinine parameters at the Specialist Hospital, Sokoto.

HISTOPATHOLOGICAL ASSESSMENT

The tissues (heart, liver, kidney and spleen) which were fixed were dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax melting at 60°C. The tissues were then cut into sections of 4–5 µm thick, stained with haematoxylin-eosin and observed under the microscope (Olympus BX41) at a magnification power of 100x.

STATISTICAL ANALYSIS

Results were expressed as the mean ± standard error of the mean. The difference between groups was determined by one-way analysis of variance (ANOVA) using statistical package for social sciences (SPSS, version 20.0) software for windows. *Post-hoc* test (Dunnett Multiple Comparison) for inter-groups was applied. Significance was considered at $p < 0.05$.

RESULTS

ACUTE TOXICITY STUDIES

The result of the acute toxicity is shown in Table 1. No mortality in both phases I and II was recorded.

HAEMATOLOGICAL INVESTIGATION

The effect of the aqueous leaf extract of *L. taraxacifolia* after 28 days of continuous oral administration showed a dose-dependent increase in WBC, RBC and Hb concentrations. The RBC count at 1000 mg/kg of the extract showed a statistically significant increase ($P < 0.05$). No statistically significant increases were recorded for the mean cell volume (MCV) as well as platelets count in the treatment groups versus control ($P < 0.05$; Figure 1).

RENAL FUNCTION INDICES

The effects of the *L. taraxacifolia* extract after 28 days of oral administration on the animal's electrolytes showed a dose-dependent increase in sodium ions which was statistically significant in the treatment groups versus control. There was a significant decrease in potassium ion concentrations in both 250 and 500 mg/kg does when compared to the control ($P < 0.05$) while the 1000 mg/kg caused a significant increase in potassium ion concentration ($P < 0.05$). The urea and bicarbonate concentrations were lower in the treatment groups versus control, while the chlorine levels were higher in the treated than the control (Table 2).

LIVER FUNCTION INDICES

The results showed a net reduction in total bilirubin (TB) and alkaline phosphatase (ALP) in all treatment groups. Increases

Table 1 Oral acute toxicity of aqueous extract of *Launaea taraxacifolia* in rats.

Oral dose (mg/kg)	Number of rats dead/ Number of rats used	% mortality
PHASE I		
10	0/3	0
100	0/3	0
1000	0/3	0
PHASE II		
1600	0/1	0
2900	0/1	0
5000	0/1	0

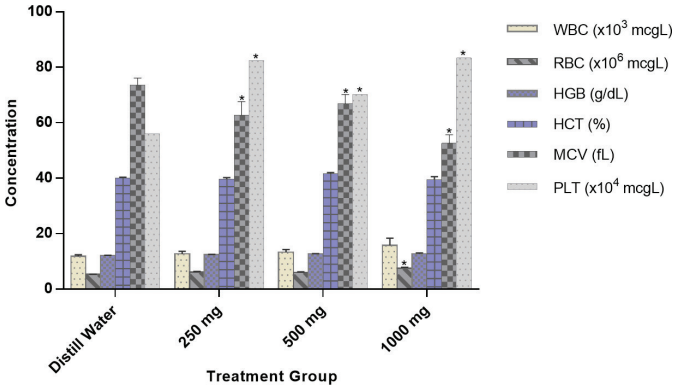

Figure 1 Effect of aqueous leaf extract of *Launaea taraxacifolia* (Willd) on haematological indices following 28 days of oral treatment in Wistar rats. *Statistically significant at P > 0.05.

Table 2 Effect of aqueous leaf extract of *Launaea taraxacifolia* (Willd) on renal function indices following 28 days oral treatment in Wistar rats.

Treatment group (mg/kg)	Urea (mmol/L)	Creatinine (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)	Chlorine (mmol/L)	Bicarbonate (mmol/L)
Distilled water	6.42 ± 0.17	1.12 ± 0.05	148.20 ± 1.35	5.72 ± 0.14	108.60 ± 1.20	25.60 ± 0.50
250	6.20 ± 0.10	1.26 ± 0.30	153.87 ± 0.58*	5.20 ± 0.07*	116.80 ± 0.96	25.00 ± 0.70
500	6.18 ± 0.80	1.08 ± 0.15	155.40 ± 0.50*	5.28 ± 0.12*	111.60 ± 2.15	25.40 ± 0.50
1000	6.34 ± 0.11	1.12 ± 0.06	162.80 ± 0.66*	6.46 ± 0.08*	112.00 ± 1.92	25.40 ± 1.02

* Statistically significant at P > 0.05.

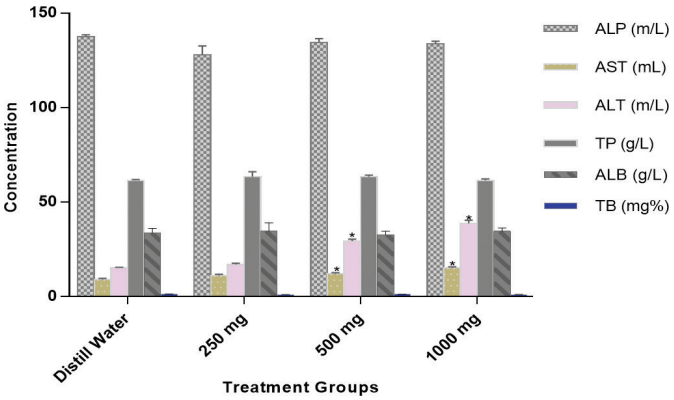

Figure 2 Effect of aqueous leaf extract of *Launaea taraxacifolia* (Willd) on liver function indices following 28 days of oral treatment in Wistar rats. *Statistically significant at P > 0.05.

in aspartate transaminase (AST), alanine transaminase (ALT) and total protein versus control was recorded. Both AST and ALT were dose-dependent and significant (P > 0.05) at 500 and 1000 mg/kg. The albumin (ALB) showed a slight increase for the 250 and 1000 mg/kg, but a reduction for the 500 mg/kg versus control (Figure 2).

HISTOLOGICAL FINDINGS

Processed tissues in the kidney showed normal glomeruli, tubules and interstitium in all treatment groups (Figure 3). The liver assessment showed that all processed tissues had a normal portal triad, central vein and hepatocytes (Figure 4). The same result was observed in the heart tissues, as all treatment groups showed normal cardiac myocytes. However, microscopic examination of the spleen showed normal lymphoid follicles for the 250 mg/kg group. But sections of the groups treated with 500 and 1000 mg/kg showed follicular hyperplasia, congestion and oedema represented by a black arrow in Figure 5 (plate C and D, respectively).

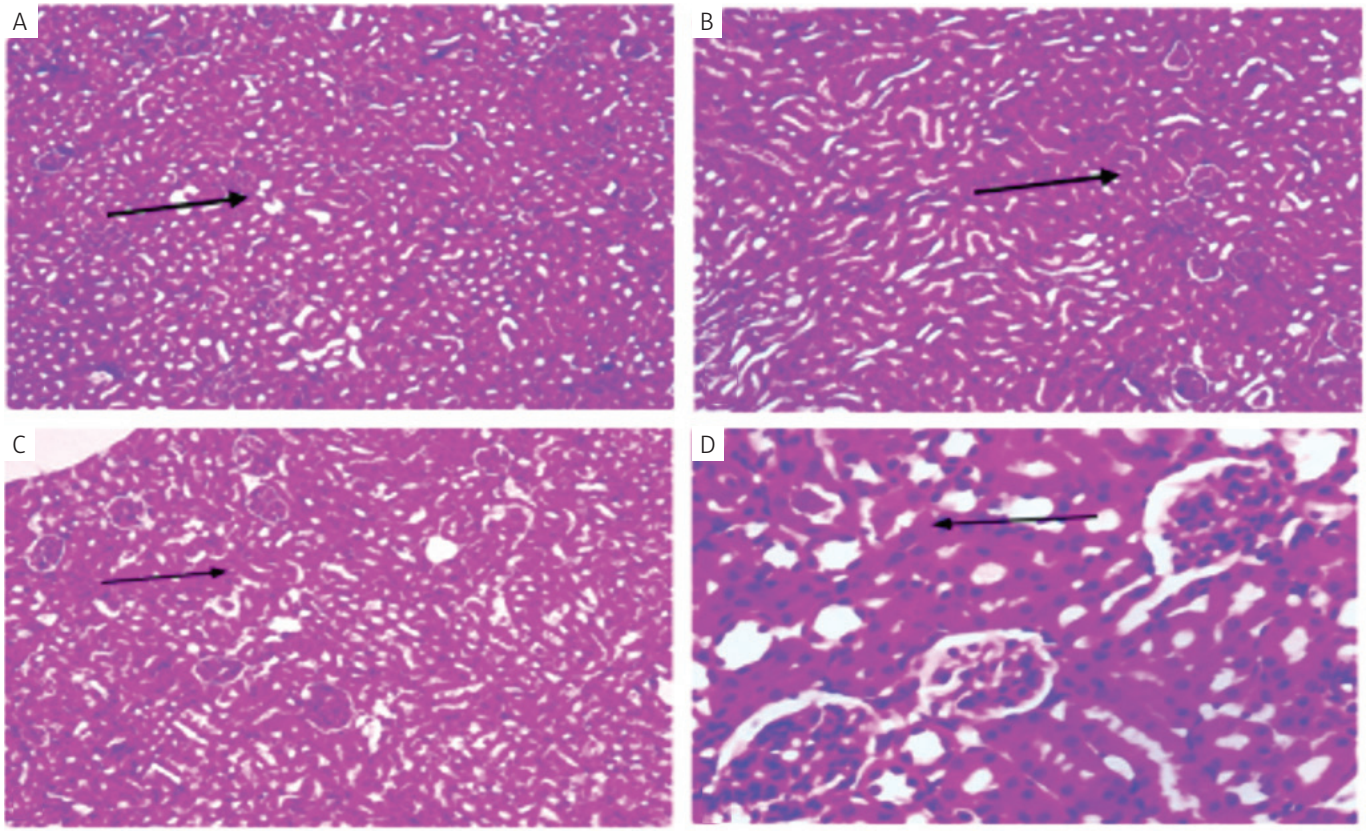

Figure 3 Photomicrograph of a section of rat kidney treated with *Launaea taraxacifolia* (Willd) following 28 days of oral administration (H,E; 100x), distilled water (A),250 mg/kg (B), 500 mg/kg (C) and 1000 mg/kg (D) extract treated groups.

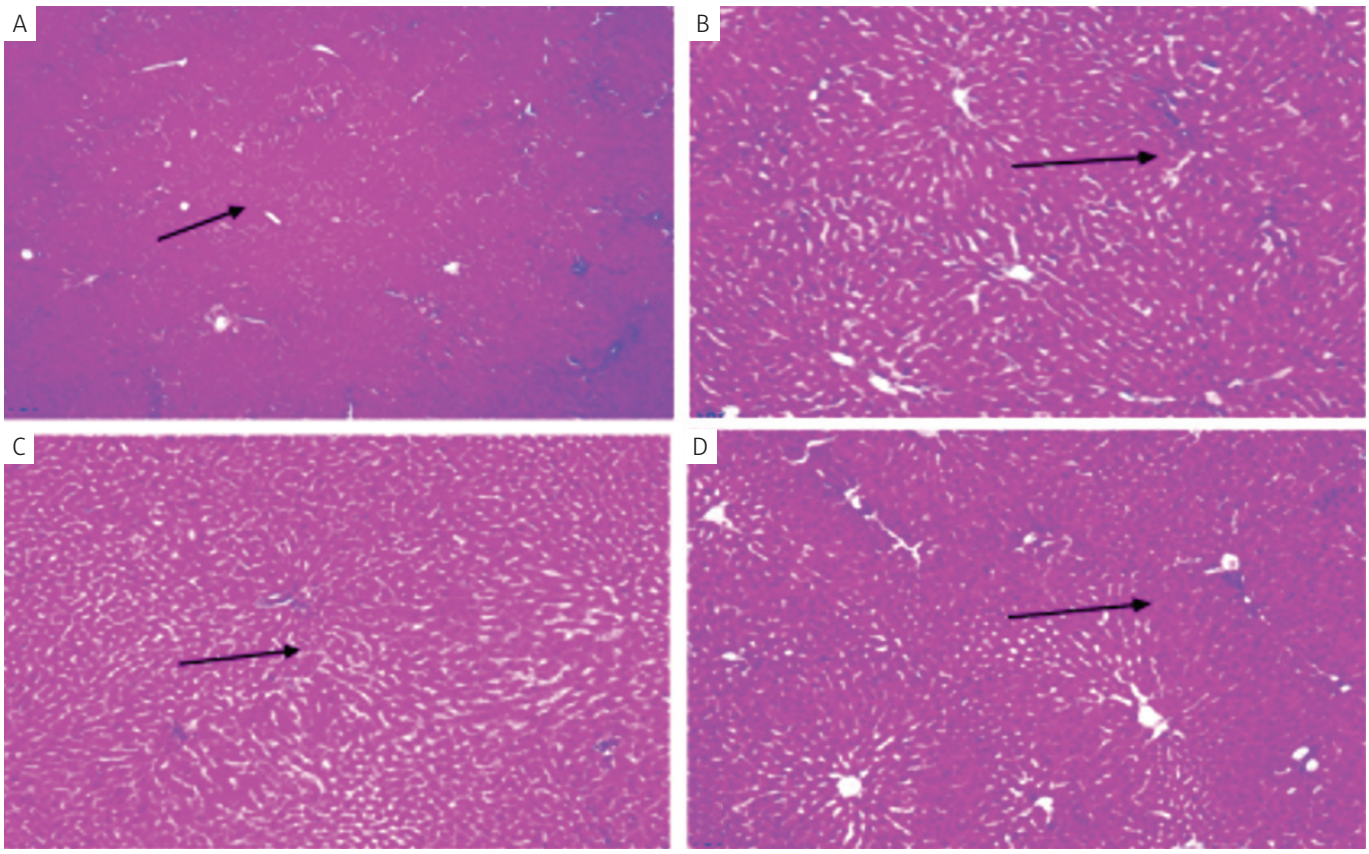

Figure 4 Photomicrograph of a section of rat liver treated with *Launaea taraxacifolia* (Willd) following 28 days of oral administration (H and E, 100x) distilled water (A),250 mg/kg (B), 500 mg/kg (C) and 1000 mg/kg (D) extract treated groups.

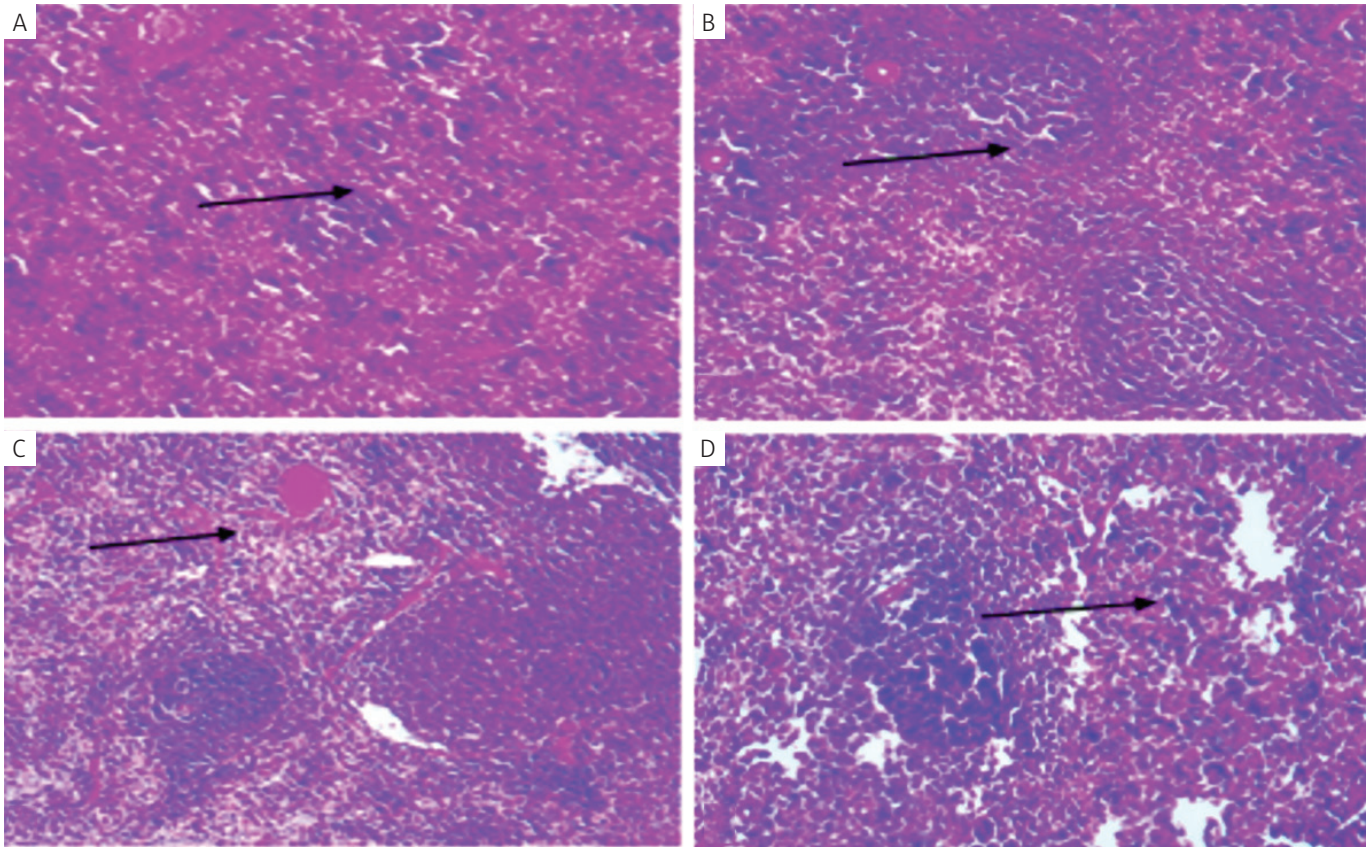

Figure 5 Photomicrograph of a section of rat spleen treated with *Launaea taraxacifolia* (Willd) following 28 days of oral administration (H and E, 100x), distilled water (A),250 mg/kg (B), 500 mg/kg (C) and 1000 mg/kg (D) extract treated groups.

DISCUSSION

The top priority of any government is to provide food, security and health benefits for its citizens.[7] The bioactive metabolites produced by plants are used as medicines for decades.[3,16–18]

L. taraxacifolia is a herbal plant with economic and ethnobotanical potentials, and is easily domesticated.[6,9] Several studies have investigated the nutritional and medicinal benefits of this plant and have reported that it contains flavonoids, saponins, phenol, ascorbic acid, phenol, oxalate and minerals such as calcium, manganese, selenium, potassium, magnesium, and zinc.[6,19,20] The presence of the flavonoids and ascorbic demonstrate a strong antioxidant potential of the plant that if consumed, will over time help in evading off free radicals and also improve normal physiological and biological processes.[5,16,21]

The acute toxicity of *L. taraxacifolia* showed no mortality in animals when used up to a dose of 5000 mg/kg of the extract. At this dose, a plant is usually presumed safe for oral consumption.[15,17] In the 28 day's sub-acute toxicity study, no observable behavioural or toxic effects to the animals because of the administered extract indicates the relative safety of *L. taraxacifolia* throughout the study, even at the high dose of 1000 mg/kg.

The effect of *L. taraxacifolia* after the 28 days of oral administration showed a dose- dependent increase in WBC and RBC. Although this was not statistically significant except for RBC at the highest dose of 1000 mg/kg, suggesting a possible stimulation of erythropoietin.[22-23] The increased WBC can be an indication of the immunity-boosting property of the plant. There was no significant change in Hb or HCT concentration. But a statistically significant increase in platelets count agreed with a previous study by Tohti *et al*.[24] The increase in platelets could have been due to the presence of some phytochemicals such as saponins and cardiac glycoside.[24–26]

The three divided doses of *L. taraxacifolia* used in this study (250, 500 and 1000 mg/kg) showed no significant changes in the urea, creatinine or carbonate levels in the animals following the 28 days of oral administration. Serum urea accumulation was used as a marker for acute renal injury, and serum creatinine accumulation was used in detecting chronic renal toxicity.[13,27] The values of both creatinine and urea were lower or comparable with the control, pointing to the safety of *L. taraxacifolia* with regards to the kidneys, and histological findings showed no pathologies in the tissues examined. However, there was a statistically significant dose-dependent increase in sodium ion concentrations and a non-uniform effect on the potassium concentrations as a dose-dependent decrease was observed for 250 and 500 mg/kg groups, respectively, but a significant increase for the 1000 mg/kg dose. That indicated that the extract of *L. taraxacifolia* of different doses had minimal effect on the body's electrolytes but must be taken with caution in people with hypertension, potential for hypertension and prolonged extract use. The levels of AST, ALT and ALP are biomarkers for liver function or injury. The result of this study reveals a significant increase in the level of AST and ALT, which was statistically significant at 500 and 1000 mg/kg doses indicating the possible hepatotoxic effect of the extract when compared with the control group, but there was a net decrease in ALP. The histology investigation of the rat's liver cells revealed no damage at the cellular level. The increases in AST and ALT was an indication of possible liver inflammatory potentials of *L. taraxacifolia* on chronic dosing longer than 28 days, although the presence of flavonoids and phenols compounds could mitigate this unwanted effect. Extract of LT has also been shown to normalized the lipid profile.[29]

Histological examination of the tissues showed no cellular damage, except for the spleen tissue, indicating that *L. taraxacifolia* leaf extract can be relatively safe at low doses. Similar findings of an increase in serum concentration of ALT and ALP were reported by Peter *et al*.[28]

The study of Isehunwa *et al*.[4] showed that a dose of 200 mg/kg *L. taraxacifolia* extract significantly decreased the blood glucose and liver glycogen and increased muscle glycogen and lactate dehydrogenase activity after one week of administration, but was not seen post two weeks.[4,30]

CONCLUSION

The acute and sub-acute toxicities of the leaf extract of *L. taraxacifolia* was evaluated orally and found to be safe. This study supports the use of *L. taraxacifolia* as a potential medicine and food. So the authors recommend the use and cultivation of the plant as a potential source for food security in the African continent.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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