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Full Length Research Paper

Pharmacognostic and toxicological evaluation of the leaves of *Piper guineense* Schum. and Thonn (Piperaceae)

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The leaves of *Piper guineense* Schum. and Thonn (Piperaceae) are used in ethnomedicine for the management/treatment of various diseases. The objective of this study was to establish the pharmacognostic and toxicological profiles of *P. guineense* to help practitioners in making choices, and to assist scientists involved in the research of the *Piper* genus. The fresh leaves were examined for their macroscopic and microscopic properties. Numerical (quantitative analysis) and phytochemical evaluations were carried out using standard methods. Acute toxicity profile of the plant, including LD 50 was investigated using mice while sub-acute toxicity to determine effects of the plant leaf extract on some major organs was investigated for 30 days in rats. The macroscopic, microscopic and numerical features observed in the leaves of *P. guineense* could aid in sample identification. Glycosides, alkaloids and phenolics were among the secondary plant metabolites present. Oral doses ≤ 8000 mg/kg did not lead to death of the animals. Sub-acute toxicological evaluations at the doses of 250 and 500 mg/kg showed mild congestion in all the target organs, except spleen, where at 500 mg/kg, in addition to mild stromal oedema, there was mild follicular activation and moderate hyperplasia of sinus histiocytes. These results could aid researchers and practitioners in the investigations and consumption of the leaves of *P. guineense*. Its overall safety profile needs to be further evaluated and care should be taken on prolonged usage.

Key words: *Piper guineense*, Piperaceae, pharmacognostic standardization, toxicological.

INTRODUCTION

The standardization of herbal medicines is the process involving a series of laboratory experiments that reveal and assemble a set of inherently peculiar characteristic. These characteristics include constant parameters that are definitive, with qualitative and quantitative values or specific, unique and unshared features on the basis of which similar herbal medicines, claimed to be the same, can be compared for the purpose of authenticity, genuineness, purity, efficacy, safety, repeatability, reproducibility and the overall quality assurance (Elujoba,

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1999).

Klein (1996) stated that medicinal plants have the potential to be harmful, postulating that it is dependent on the dose of substance consumed. However, probably because medicinal plants are natural, a greater proportion would have to be consumed to cause harm when compared with orthodox drugs. Therefore, determination of acute and sub-acute toxicity profiles of medicinal plants is essential to guide herbal medicine practitioners and researchers (Abere et al., 2014).

Piper guineense Schum. and Thonn belongs to the Piperaceae family (Macmillan, 1984). Common names include African black pepper, Benin pepper, Guinea cubeb pepper or Ashanti pepper. It is also known as uririe (Urhobo), Iyere (Yoruba) and Uziza (Ibo). It is native to the tropical and subtropical world (Nwinyi et al., 2009). It is a highly valued ingredient in Africa because of its varied and numerous uses. The ethanol leaf extract of *P. guineense* has significant anti-inflammatory and diuretic properties (Omodamiro and Jimoh, 2014). Udoh et al., (2012) reported that consuming the leaves of *P. guineense* have the ability to interfere with the female reproductive system by affecting the activities of the uterine muscles. The antifertility effects of the aqueous extract on wistar rats had earlier been reported by Mbongue et al. (2005). Ekanem et al. (2010) had attributed these effects to the activities of alkaloidal amides on the uterine muscles. The piperine-amide present in the leaf is responsible for its insecticidal activity against *Zonocerus variegatus* (Nwinyi et al., 2009). Antiparasitic, antimicrobial and antifungal activities of the leaves and seeds of *P. guineense* have also been reported (Ekanem et al., 2010).

In view of the challenges confronting the practice of herbal medicines, provision of information on the toxicity of these products will assist and encourage herbal medicine practitioners and researchers (Adefemi et al., 1988). This study on the leaves of *P. guineense* was geared towards establishing its pharmacognostic and toxicological profiles to aid in differentiating it from similar species.

MATERIALS AND METHODS

Preparation of plant extract

P. guineense Schum. and Thonn (Piperaceae) leaves were collected in Benin City, Nigeria. Authentication was done by the plant curator at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City where a voucher specimen was lodged. The leaves were dried at ambient temperature for 72 h and powdered.

Animals

Albino mice of Swiss origin (21. 44 ± 1. 02 g) and Wistar male rats (239. 00 ± 14. 02 g) were sourced from the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. Animals used for the experiments were handled with care and procedures duly followed international standards for use of animals in experiments (National Institute of Health, 2002). The ethical approval of the study was part of the comprehensive work design approved by the College of Medicine, University of Benin Animal Ethics Committee (ADM/F. 22A/Vol. viii/349).

Macro- and micro-morphological examinations

The macroscopic and microscopic chemomicroscopic examinations of the fresh and powdered leaves of *P. guineense* were carried out using standard methods (Wallis, 1985; Evans, 2006; AP, 1986).

Phytochemical investigation

Screening for secondary plant metabolites was carried out according to previously described methods (Brain and Turner, 1975; Ciulei, 1981; Evans, 2006; Harborne, 1992). These include chemical tests for tannins, flavonoids, alkaloids, cardiac, saponin, anthracene and cyanogenetic glycosides.

Numerical values determinations

This involved the quantitative investigations to determine moisture content, ash values as well as solubility profiles (AP, 1986; BP, 1980).

Toxicological evaluation

Acute toxicity test

The animals (Swiss albino mice) were divided into 9 groups of 5 animals each. They were orally administered the extracts of *P. guineense* at doses range of 1-8 g/kg. Mice administered 5 ml/kg of normal saline served as the control group. On day 1, the animals were observed for 10 min for every 6 h and for at least 10 min for the next two days. Lethality as well as gross toxicological features was recorded daily for each group. These features include state of hyperactivity, diarrhea, convulsion and pile-erection (Dietrich, 1983). Observation of the animals was continued for the next fourteen days.

Sub-acute toxicity test

In the test to determine the effects of administering the extract of *P. guineense* on key organs in Wistar rats, the procedure of Abere et al. (2015) was adopted using oral doses of 250 and 500 mg/kg per day of the extract for 30 consecutive days. 5 ml/kg of normal saline (vehicle) served as the control. At day 30, the rats were sacrificed with the hearts, livers, spleen and kidney removed and preserved in 10% formaldehyde solution. The organs were sectioned (6 µ thick) and embedded in paraffin wax and stained with H&E (Adefemi et al., 1988).

RESULTS

Macroscopic characteristics of the leaves of *P. guineense*

It was a simple leaf with alternate petioles. The shape

Table 1. Numerical data of leaves of *P. guineense.*

Parameter	Mean ± SEM (% w/w)
Moisture content*	12.50 ± 0.21
Total ash*	7.9 ± 0.11
Acid – insoluble ash†	0.89 ± 0.01
Water – soluble ash†	4.10 ± 0.25
Alcohol – soluble extractive*	8.00 ± 0.08
Water – soluble extractive*	5.80 ± 0.16

*n = 30; † n = 15.

Table 2. Phytochemical constituents of *P. guineense* leaves.

Classes of secondary metabolites	Inferences
Tannins	+
Flavonoids	+
Alkaloids	+
Anthracene derivatives	-
Saponin glycosides	+
Cardiac glycosides	+
Cyanogenetic glycosides	-

-= absent; + = present.

was elliptical, venation was pinnate while apex was acuminate. Fresh leaves were greenish in colour, though darker in the upper surface. Leaf size was 10.3 cm ± 0.5 (length) and 6.0 cm ± 0.2 (breadth). The fresh leaves had an aromatic odour and a hot and very pungent taste.

Microscopic characteristics of the leaves of *P. guineense*

Microscopical examination of the leaves of *P. guineense* showed that anticlinal walls were straight and contained numerous calcium oxalate crystals scattered all over the surface of the lamina. Each stoma was surrounded by 2 subsidiary cells with their common walls at right angles to the long axis of the guard cells (diacytic arrangement). The transverse section of *P. guineense* leaf revealed an upper and lower epidermi consisting of cells that are similar in sizes. *P. guineense* had a bifacial surface, that is, the two surfaces were not identical structurally, thus the arrangement was dorsiventral. The mesophyll consisted of 2 parts, viz, the spongy mesophyll containing a crystal sheath (clusters crystals of calcium oxalate) and the palisade mesophyll. The vascular bundle, consisting of xylem and phloem tissues was surrounded by layers of fibres.

Chemomicroscopic characteristics of the leaves of *P. guineense*

Chemomicroscopic examination of *P. guineense* leaves showed the presence of starch, lignin, calcium oxalate crystals, tannins and cellulose.

Numerical values of *P. guineense* leaves

The results of the quantitative analysis of *P. guineense* leaves are presented in Table 1.

Phytochemical screening

The phytochemical investigations of *P. guineense* leaves showed the presence of tannins, flavonoids, alkaloids, saponins and cardiac glycosides (Table 2).

Toxicological evaluation

In the acute toxicity study, *P. guineense* extract caused no death up to the oral dose level of 8000 mg/kg body weight in mice, thus the LD 50 was < 8g/kg. The animals

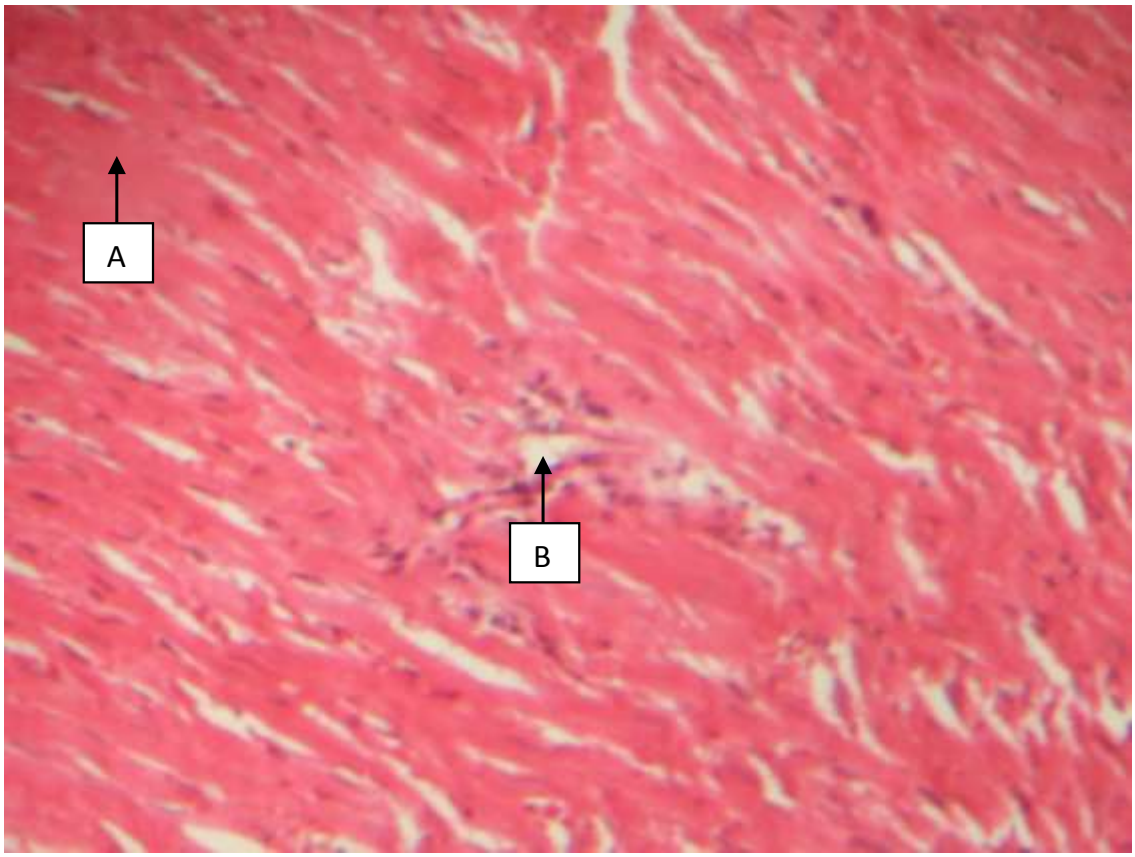

Figure 1. Photomicrograph of the heart of rats administered with normal saline for 30 days showing myocardium A, pierced by small cardiac vein B (H&E 10x).

did not show any significant changes in their behaviour, mood, movement, agility and posture. The frequency of stooling was normal. There was absence of wet stools; rather, stools were dry and rounded. The animals did not show signs of convulsion or exhibit writhing.

Administration of the extract of *P. guineense* for 30 days in rats did not produce any major toxicological symptoms nor were deaths recorded. General histopathological analysis of the heart (Figure 2) in the 250 mg/kg *P. guineense* treated group showed mild transmural oedema, vascular congestion and infiltrates of chronic inflammatory cells. There was no difference at the dose of 500 mg/kg (Figure 3). In the liver, at 250 and 500 mg/kg (Figures 5 and 6), there were mild portal vascular congestion and hypertrophy, in addition to mild periportal infiltrate of chronic inflammatory cells. The spleen at 250 mg/kg showed mild stromal oedema and mildly activated sinus histiocytes (Figure 8) while at 500 mg/kg (Figure 9), in addition to mild stromal odema and follicular activation, there were also moderate hyperplasia of sinus histiocytes. The kidney showed mild interstitial vascular congestion and hypertrophy (Figures 11 and 12). Figures 1, 4, 7 and 10 served as controls for the heart, liver, spleen and kidney' at the end of the result for toxicological evaluation.

DISCUSSION

Piper guineense is very similar to other species of the Genus Piper, especially *Piper nigrum*, (pepper of commerce) which is the source of black and white peppers. The Piperaceae family has well over 700 species (Nwinyi et al., 2009). The presence, nature and spread of pharmacognostic characters in medicinal plants aid in their identification. It is pertinent to note that the establishment of pharmacognostic parameters and standards of a medicinal plant are important before it can be included in an Herbal Pharmacopoeia (Prajapati and Patel, 2010). The morphological features described for the leaves of *P. guineense* could therefore, serve as a basis for the identification and further investigation of the plant.

The numerical values of the leaves of *P. guineense* described could assist in establishing the true identity of the plant. The moisture content of 12.5% for the leaves of *P. guineense* is within the pharmacopoeia limits of water content for vegetable drugs given as 8 to 14% (AP,1986).

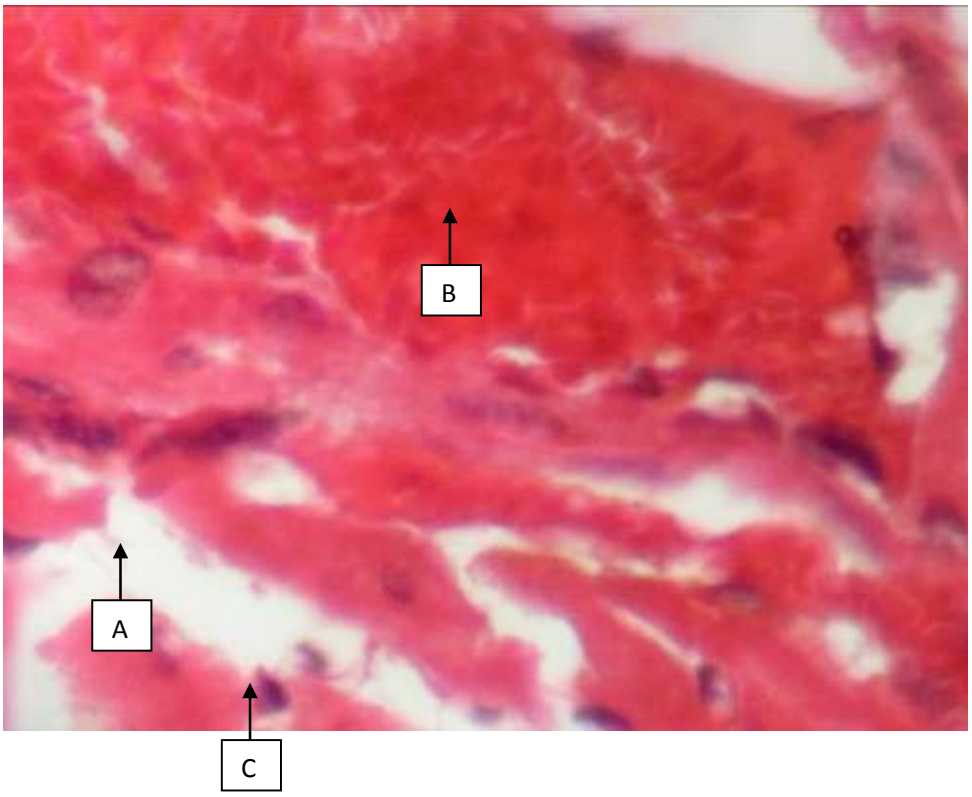

Figure 2. Photomicrograph of the heart of rats administered with 250 mg/kg extract of *P. guineense* for 30 days showing mild transmural oedema A, mild vascular congestion and dilatation B and mild infiltrate of chronic inflammatory cells C (H&E x 40).

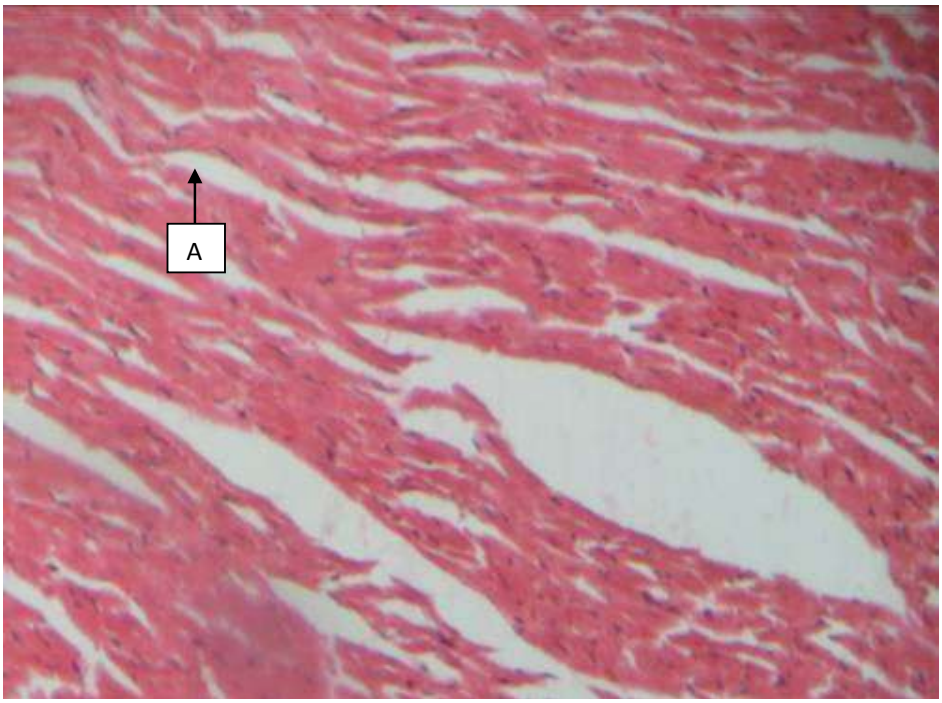

Figure 3. Photomicrograph of the heart of rats administered with 500 mg/kg extract of *P. guineense* for 30 days showing mild transmural oedema A (H&E x 10).

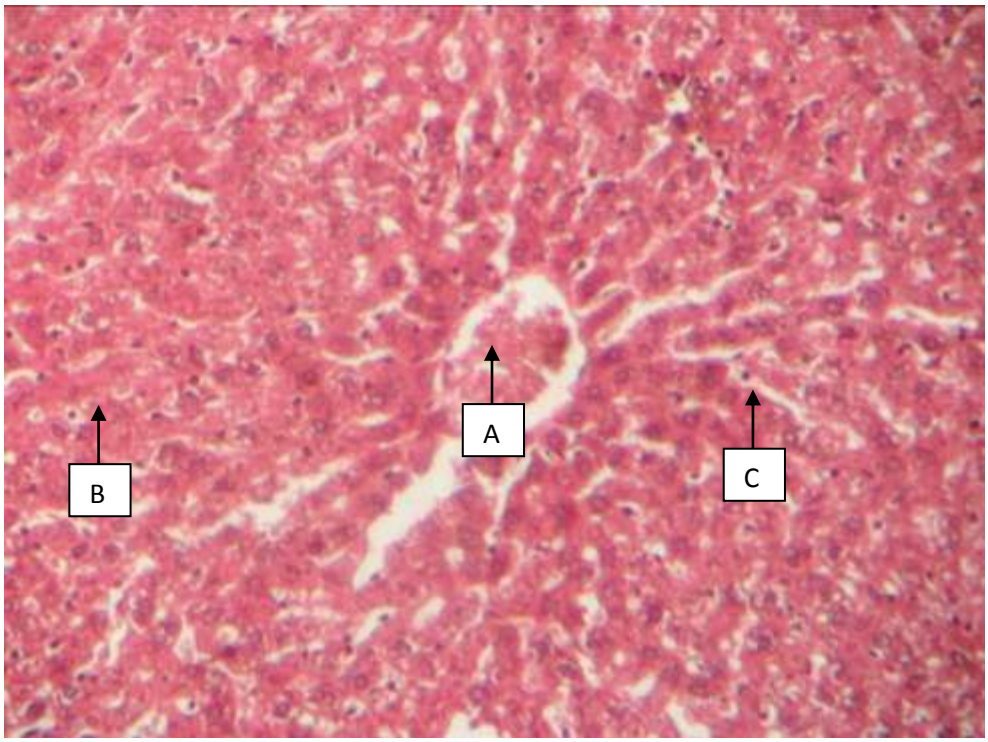

Figure 4. Photomicrograph of the liver of rats administered with normal saline for 30 days showing central vein A, hepatocytes B and sinusoids C (H&E x 10).

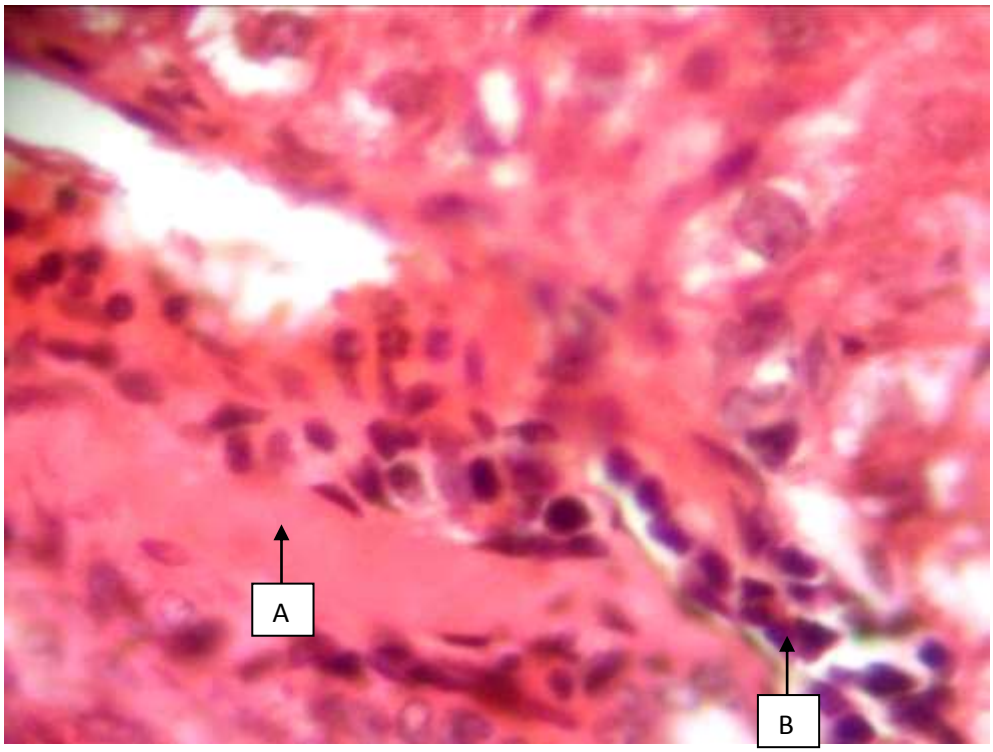

Figure 5. Photomicrograph of the liver of rats administered with 250 mg/kg extract of *P. guineense* for 30 days showing mild portal vascular congestion and hypertrophy A and mild periportal infiltrate of chronic inflammatory cells B (H&E x 40).

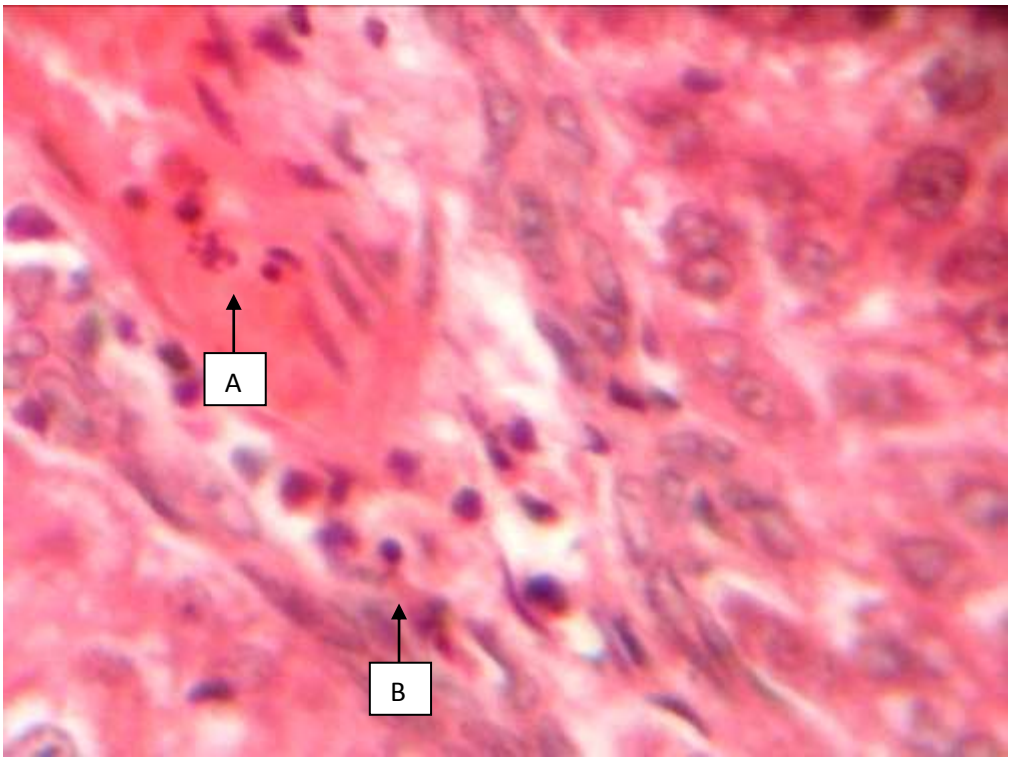

Figure 6. Photomicrograph of the liver of rats administered with 500 mg/kg extract of *P. guineense* for 30 days showing mild portal vascular congestion and dilatation A and mild infiltrates of chronic inflammatory cells B (H&E x 40).

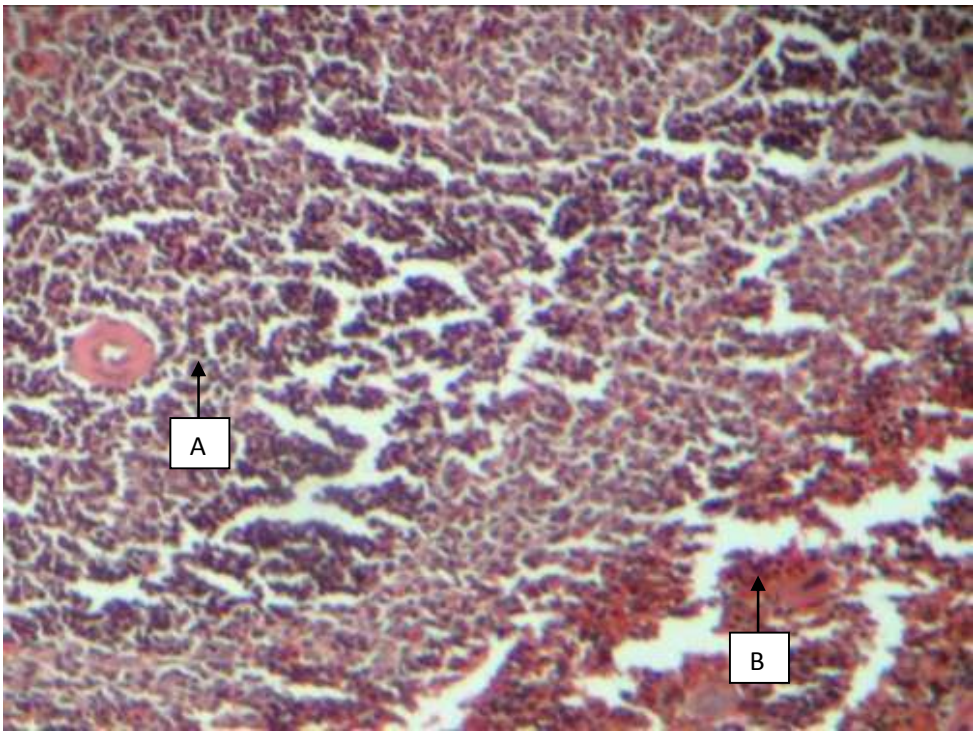

Figure 7. Photomicrograph of the spleen of rats administered with normal saline for 30 days showing white pulp A and red pulp B (H&E x 10).

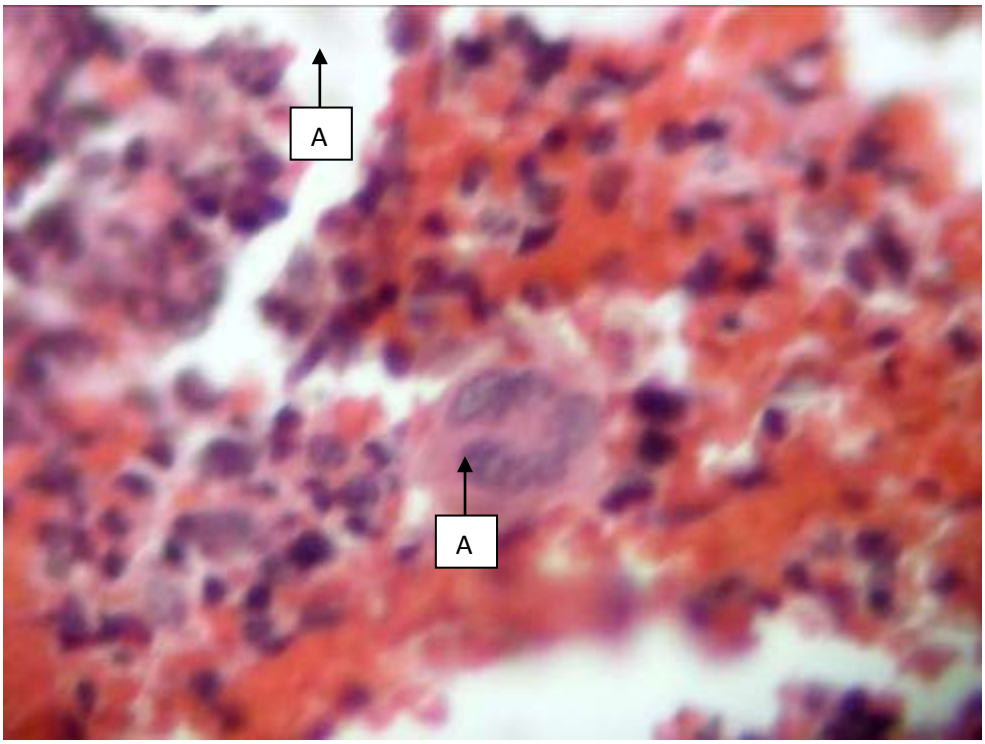

Figure 8. Photomicrograph of the spleen of rats administered with 250 mg/kg extract of *P. guineense* for 30 days showing mild stromal oedema A and mildly activated sinus histiocytes B (H&E x 40).

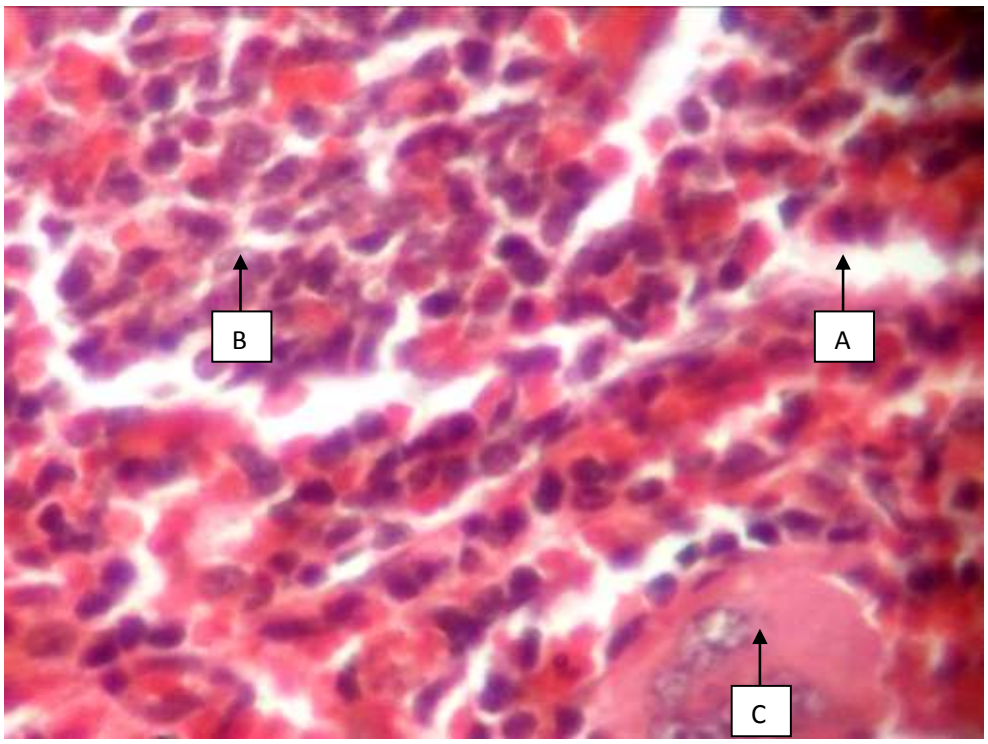

Figure 9. Photomicrograph of the spleen of rats administered with 500 mg/kg extract of *P. guineense* for 30 days showing mild stromal oedema A and mild follicular activation B and moderate hyperplasia of sinus histiocytes C (H&E x 40).

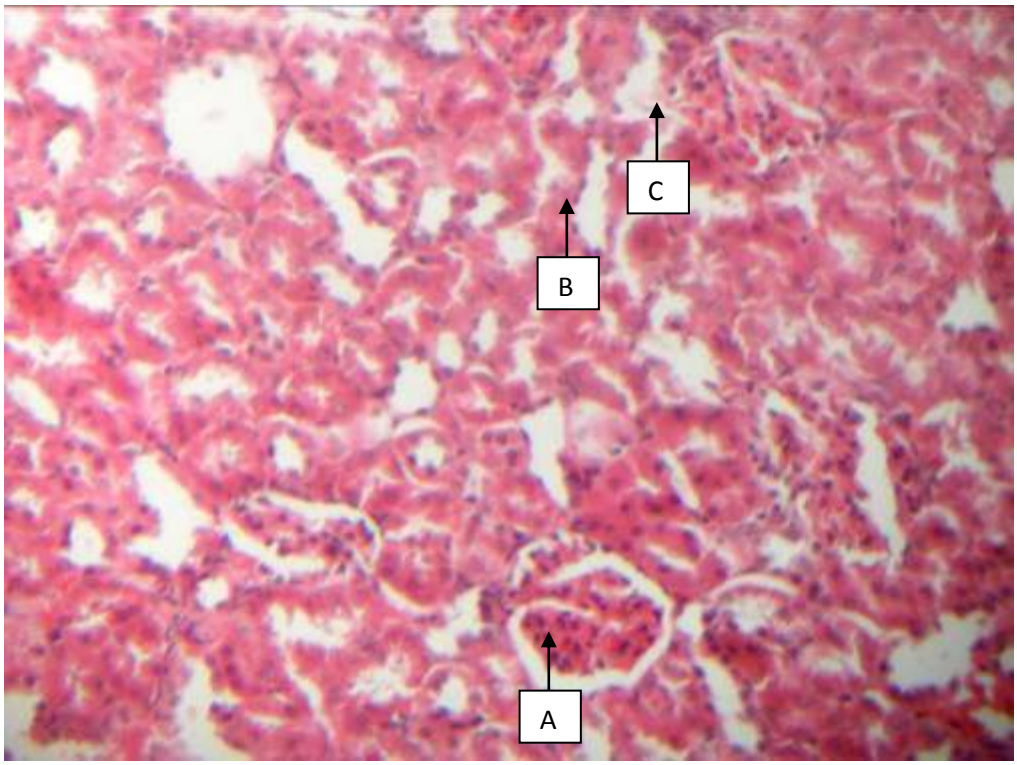

Figure 10. Photomicrograph of the kidney of rats administered with normal saline for 30 days showing glomeruli A and tubules B separated by interstitial space C (H&E x 10)

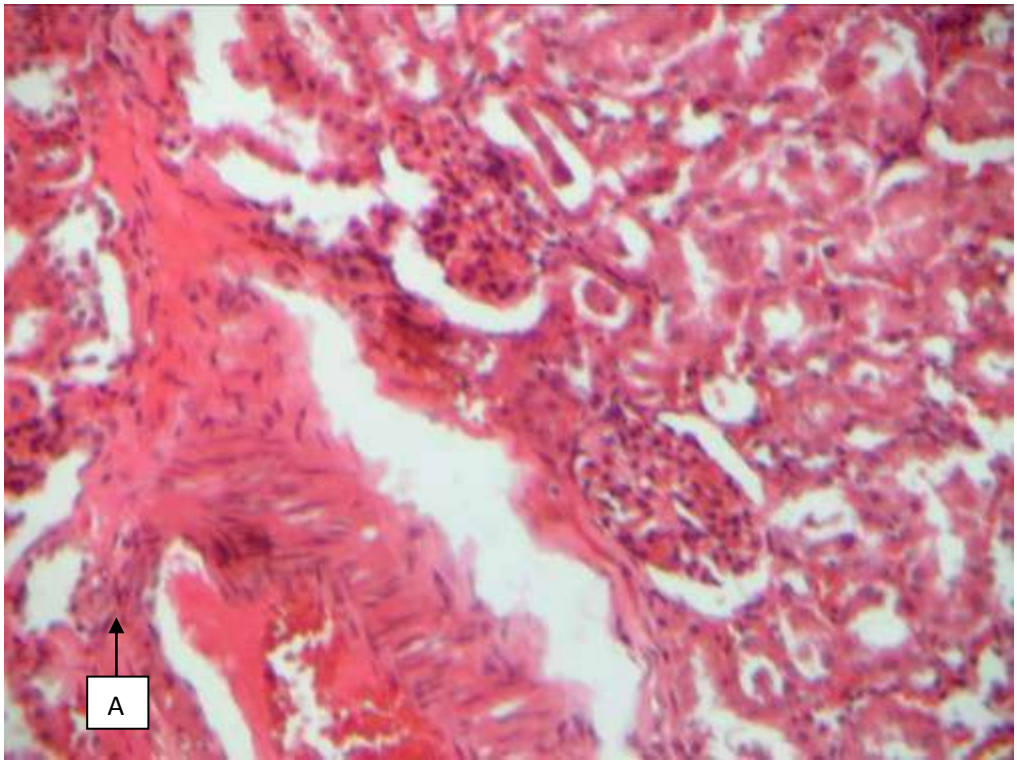

Figure 11. Photomicrograph of the kidney of rats administered with 250 mg/kg extract of *P. guineense* for 30 days showing mild interstitial vascular congestion and hypertrophy A (H&E x 10).

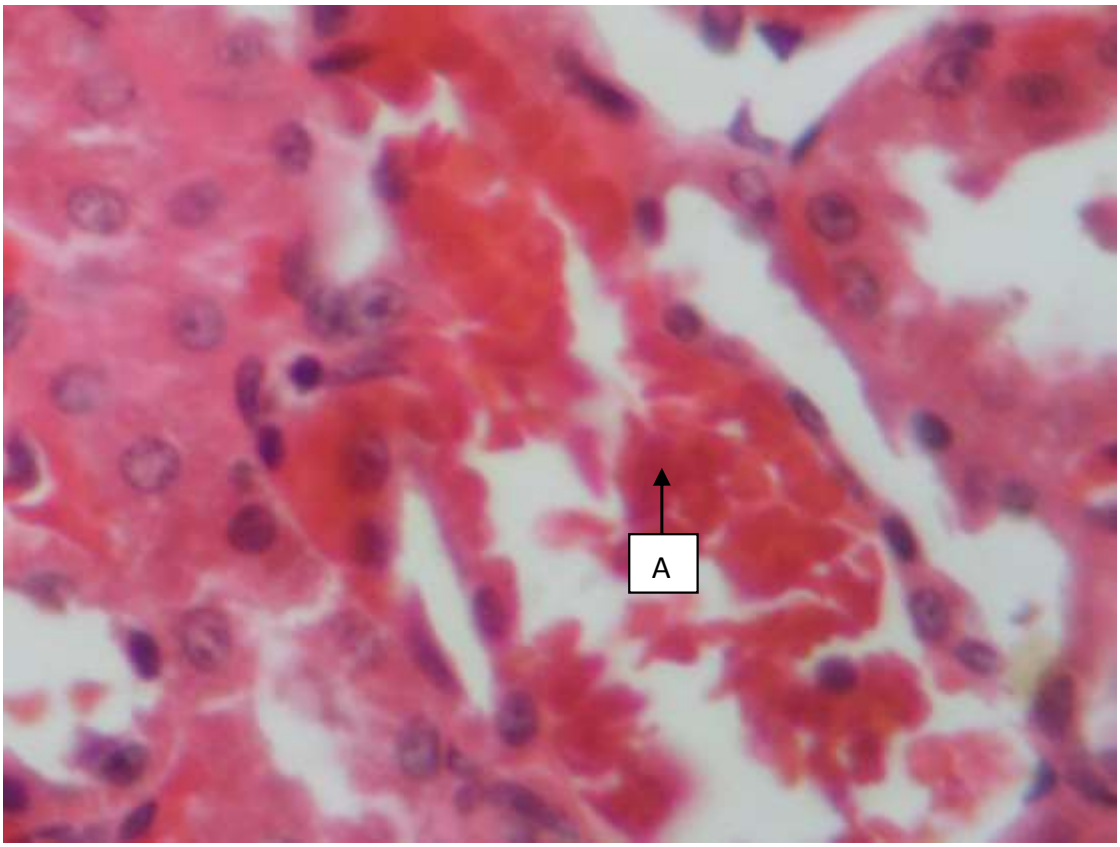

Figure 12. Photomicrograph of the kidney of rats administered 500 mg/kg extract of *P. guineense* for 30 days showing mild interstitial vascular congestion and hypertrophy A (H&E 40x).

1986). The leaves of *P. guineense* can be stored in tightly covered containers at ambient temperatures without much concern to the deterioration of the active ingredients. The values of the acid-insoluble ash (0.89 ± 0.01) and water-soluble ash (4.10 ± 0.25) gave an indication that *P. guineense* can be used directly as powdered drugs.

The administered dose of the herbal drug primarily determines its safety. Acute and sub-acute toxicological studies of herbal medications, especially the determination of the median lethal dose (LD 50) are very desirable, as this could give an index of the dose regimen. Where the herbal formulation is to be administered for more than 3 days, it becomes necessary to subject them to sub-acute toxicity test (Abere et al., 2014). The main reason is to determine the likely organs that could be susceptible to toxicity by the herbal drug on prolonged usage. Histopathological effects of the administration of 250 and 500 mg/kg per day of the extracts of *P. guineense* on rats showed no significant differences at low and high doses in the liver, kidney and heart, except with the spleen, where there was mild stromal oedema and mildly activated sinus histiocytes at the dose of 250 mg/kg. At the dose of 500 mg/kg, in addition to mild stromal oedema, there was mild follicular activation and moderate hyperplasia of sinus histiocytes. Effects on the other organs were majorly mild.

CONCLUSION

Scientific data that could aid the standardization of *P. guineense* Schum. and Thonn (Piperaceae) have been provided. Any medicinal product that is claimed to be *P. guineense,* but whose leaves have characteristic that are significantly different from the standards presented could be rejected. In the light of the toxicological results, care should be taken when using the drug for a long period.

CONFLICT OF INTERESTS

The authors have none to declare.

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