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ABSTRACT

Introduction: Picralima nitida is a seed bearing tree whose dried seeds are used in traditional medicine throughout West Africa, particularly in Ghana, Ivory Coast and Nigeria. The seeds are crushed and taken orally for the treatment of malaria, diarrhoea, pain, hypertension, jaundice, dysmenorrheal, and gastrointestinal disorders. There is paucity of data on the toxicity and safety profile of Picralima nitida, thus the need for this study. Acute toxicity study was carried out on Picralima nitida aqueous and 80% ethanolic extract in order to determine its acute toxicity and LD_{50}.

Keywords: picralima nitida, phytochemical constituents, acute oral toxicity, histopathology, medicinal plants.

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Oluwagbemiga O. Aina[α], Onyinye C. Okoyenta[σ], Kafilat O. Kareem[ρ], Damilare J. Bamgbose[Ↄ], Olusola Ajibaye[¥] & Bamgboye M. Afolabi[§]

ABSTRACT

Introduction: Picralima nitida is a seed bearing tree whose dried seeds are used in traditional medicine throughout West Africa, particularly in Ghana, Ivory Coast and Nigeria. The seeds are crushed and taken orally for the treatment of malaria, diarrhoea, pain, hypertension, jaundice, dysmenorrheal, and gastrointestinal disorders. There is paucity of data on the toxicity and safety profile of Picralima nitida, thus the need for this study. Acute toxicity study was carried out on Picralima nitida aqueous and 80% ethanolic extract in order to determine its acute toxicity and LD_{50}.

Methods: Phytochemical analysis was carried out on Picralima nitida to identify its active phytochemical constituents both qualitatively and quantitatively. Acute oral toxicity tests were done using female Swiss albino mice that weighed 20-23g following the OECD methods. Fixed doses of 300mg/kg, 2000mg/kg and 5000 mg/kg of Picralima nitida aqueous and 80% ethanolic extracts were administered to the animals once and then observed for 14days. The control group received distilled water only ad libitum. At the end of the 14days, the animals were sacrificed and analyzed for histopathological changes.

Results: The LD_{50} of the aqueous and ethanolic extract of Picralima nitida were found to be ≥2000mg/kg. The heart of the animals that received only distilled water, and those that received 300mg/kg, 2000mg/kg, and 5000mg/kg of Picralima nitida aqueous extract had no histopathological damages. The photomicrograph of the liver, kidneys, lungs of the untreated and treated groups of aqueous extract show some histopathological alterations. While, the histologic sections of the heart, liver, kidney, and lungs of the animals that received 2000mg/kg and 5000mg/kg of ethanolic extract of Picralima nitida had some histopathological changes. Similar injuries were also seen in the untreated group.

Conclusion: The phytochemical screening revealed that Picralima nitida contains important antioxidants and other phytochemicals with various health benefits. While the acute toxicity assessment of the aqueous and 80% ethanolic extracts of Picralima nitida indicate that Picralima nitida is safe.

Keywords: *picralima nitida*, phytochemical constituents, acute oral toxicity, histopathology, medicinal plants.

Author α σ ρ Ↄ ¥: Centre for Research in Traditional, Complementary and Alternative Medicine, Biochemistry and Nutrition Department, Nigerian Institute of Medical Research (NIMR), 6, Edmund Crescent, P.M.B 2013, Yaba, Lagos, Nigeria.
§: Health, Environment and Development Foundation (HEENDEF),18 Ogunfunmi Street, Surulere, Lagos, Nigeria. African, Pan-African Health and Collaborative, Salisbury, North Carolina, USA.

I. INTRODUCTION

Medicinal plants are often used in the treatment of various ailments in traditional and complementary medicine in many parts of the world [1, 2]. P*icralima nitida* (Eso Abere) is a plant genus in the family Apocynaceae, first described as a genus in 1896. It contains only one known species, *Picralima nitida*, native to tropical Africa (Benin, Ghana, Ivory Coast, Nigeria, Gabon, Cameroon, Cabinda, Central African Republic, Republic of Congo, Zaire, Uganda) [3, 4]. *Picralima nitida*, the akuamma, is a tree. The

dried seeds from this plant are used in traditional medicine throughout West Africa, particularly in Ghana as well as in the Ivory Coast and Nigeria. The seeds are crushed or powdered and taken orally, and are mainly used for the treatment of malaria, diarrhoea, pain, hypertension, jaundice, dysmenorrheal, gastrointestinal disorders [5, 6] . *Picralima nitida* seeds contain a mixture of alkaloids producing antipyretic and antiinflammatory effects along with analgesia in animal studies [7].

Most synthetic anticancer drugs that are currently available are highly expensive, elicit serious and toxic side-effects and mostly affordable by the rich and elite. Also, they are not readily available to the majority of the African population. Hence, researchers are currently working assiduously on herbal preparations and crude extracts from medicinal plants to be used wholly or as an alternative complementary therapy. This study aims to investigate the safety of P*icralima nitida* aqueous and 80% ethanolic extract in animal model through acute oral toxicity study in order to determine its toxicity characteristics and LD_{50} for subsequent *in vivo* therapeutic efficacy evaluation of P*icralima nitida* for anti-diabetics and anticancer activities in order to help validate drug candidates for clinical evaluation.

II. METHODS

2.1 Preparation of herbal extracts

Aqueous and ethanolic extracts of P*icralima nitida plant* were prepared according to standard methods by using distilled water and 80% ethanol respectively and the extracts obtained were then evaporated to dryness in electric oven at 40°C.

2.2 Phytochemical Analysis

Phytochemical analysis was carried out on P*icralima nitida* to identify the active phytochemical constituents both qualitatively and quantitatively. The phytochemical screenings were performed on the medicinal plants using standard procedures described elsewhere [8, 9].

2.3 Acute Oral Toxicity screening

Acute oral toxicity tests were done using female Swiss albino mice with an average weight of 20-23g. The toxicity study was done according to the OECD methods for acute oral toxicity [10]. They were obtained from the institutional animal house, Department of Biochemistry and Nutrition, Nigerian Institute of Medical Research (NIMR). The female mice used were nulliparous and non-pregnant. The animals were given standard animal pellets (Ladokun Feeds, Ibadan) and tap water *ad libitum*. The mice were maintained at a room temperature of 25 ±3°C and a 12 h light/dark cycle. The experimental Protocol was approved by the Institutional Animal Care and Use Committee (IACUC), Department of Biochemistry and Nutrition, Nigerian Institute of Medical Research (Ethics No. IRB/17/043).

The body weight of animals was recorded individually for calculating proper treatment dosage before the test. The doses used were 300mg/kg, 2000mg/kg and 5000mg/kg body weights (b.w) of P*icralima nitida* aqueous and 80% ethanolic extracts**.** Animals were administered once with the different doses of the extracts.The control group received distilled water only *ad libitum*. Animals were observed for signs of toxicity in the first four hours, then at thirty minutes intervals for the next twenty-four hours. Subsequently, they were observed daily for fourteen days for any delayed toxicity. Mortality, food consumption and water intake, as well as observation for general toxicity signs were monitored and recorded daily throughout the study.

2.4 Histopathological Analysis

The animals were sacrificed by cervical dislocation after blood collection. Vital organs (liver, kidneys, lungs, and heart) were removed through a midline incision in the mice's abdomen. The organs were subjected to histopathological examination. They were fixed in 10% buffered formalin, routinely processed and embedded in paraffin wax. Paraffin sections (5 µm) was cut on glass slides and stained with hematoxylin and eosin. An experienced pathologist conducted the analysis. The slides were examined under a light microscope under ×100 magnification.

III. RESULTS

3.1 Phytochemical constituents

The results of the phytochemical evaluations of P*icralima nitida* are presented in tables 1 and 2.

Table 1: Qualitative analysis report for P*icralima nitida* phytochemical constituents

Active Phytochemicals	Inference
Flavonoids	+
Terpernoids	+
Cardiac glycosides	-
Tannins	+
Phlobatanin	-
Steroid	-
Saponin	+
Alkaloids	+
Phenol	+
Anthraquinone	-

+ means positive – means negative

Table 2: Quantitative analysis report for P*icralima nitida* phytochemical constituents

Active Phytochemicals	Quantity
Flavonoids	36%
Tannins	15.08%
Saponin	51.5%
Alkaloids	20%
Phenol	45.78mg GAE/100g

+ means positive – means negative

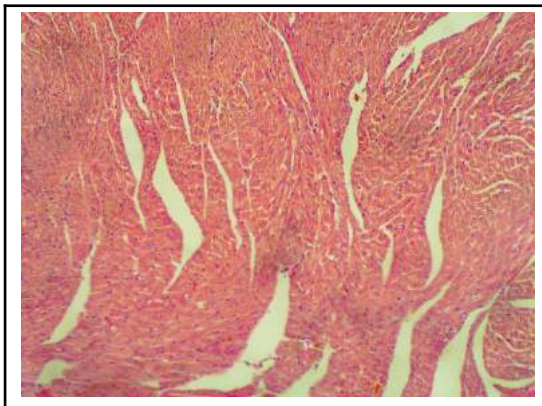
3.2 Acute Systemic Toxic effects

In this study, the LD_{50} of the aqueous and 80% ethanolic extract of P*icralima nitida* were found to be ≤ 2000mg/kg body weight.

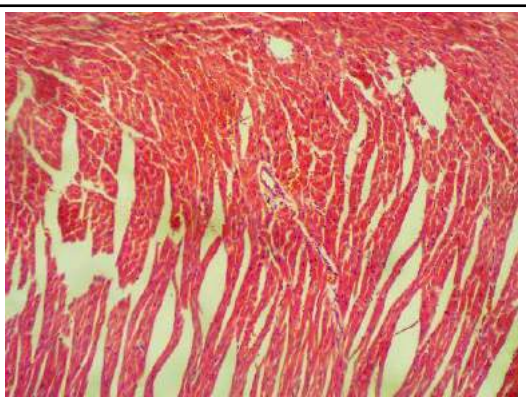
3.3 Histopathological Effect of Picralima nitida Aqueous extracts

The histologic structures of the vital organs (kidney, liver, lungs and heart) of the control group (untreated animals) and treated groups are shown in figures 1 to 4.

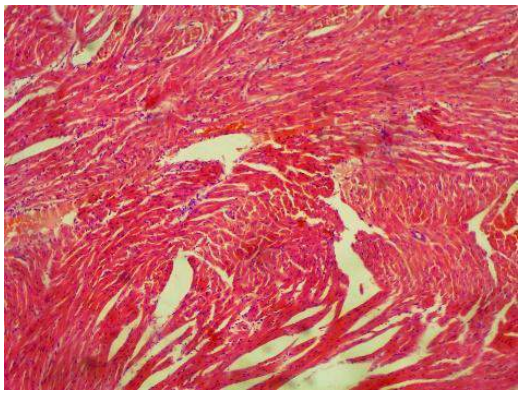
The heart of the animals that received only distilled water, and those that received low dose (300mg/kg), mid dose (2000mg/kg), and high dose (5000mg/kg) of P*icralima nitida* aqueous extract had no histopathological damages (figure 1). These indicate that the aqueous extract of P*icralima nitida* is safe on the heart even at the highest dose. However, the photomicrograph of the liver, kidneys, lungs of the untreated (control) and treated groups of aqueous extract show some histopathological alterations. The cause of the adverse effects is unknown.



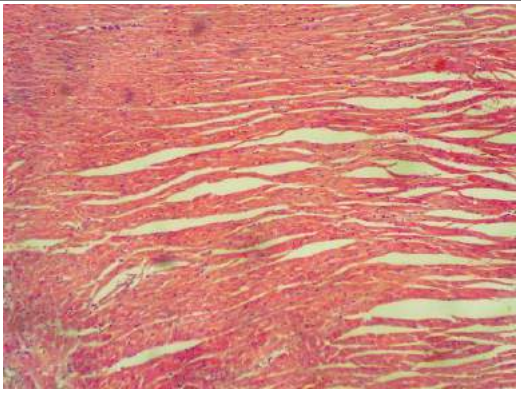
i. Control (normal)



ii. 300mg/kg (normal)

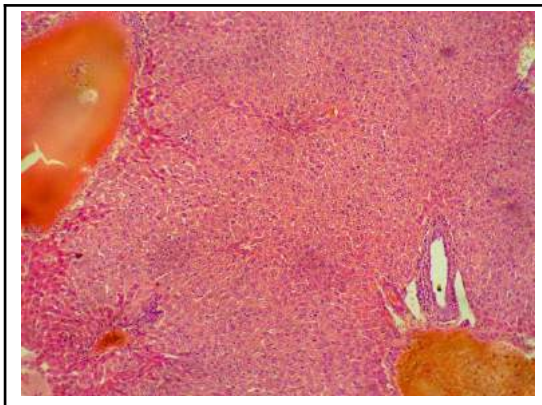


iii. 2000mg/kg (normal)

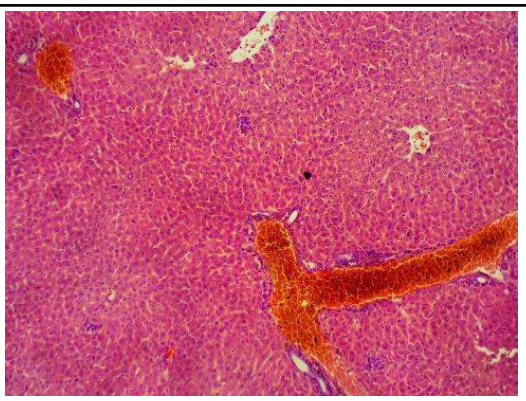


iv. 5000mg/kg (normal)

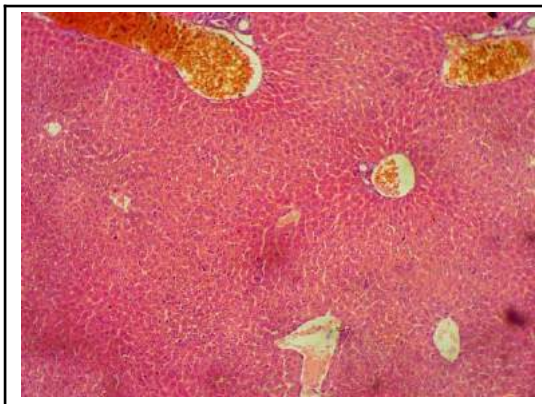
Figure 1: Histologic sections of heart excised from animals that received only distilled water (control) and those that received 300-5000mg/kg of aqueous extract of P*icralima nitida* show normal interlacing fascicles of cardiac myocytes/ myocardial cells



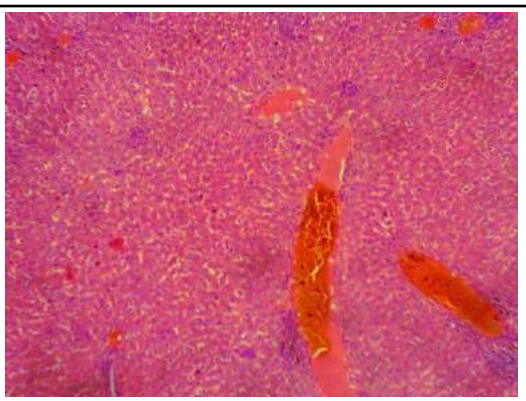
v**. Control (vascular congestion)**



vi. 300mg/kg (vascular congestion)

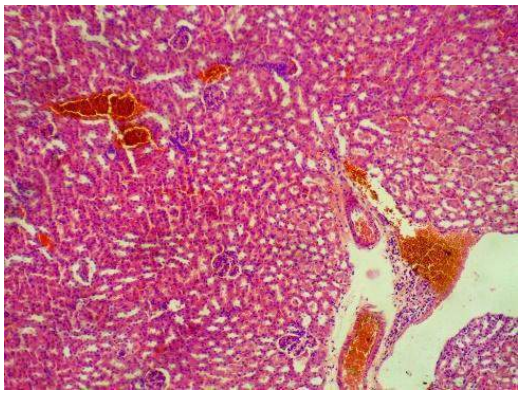


vii. 2000mg/kg (vascular congestion)

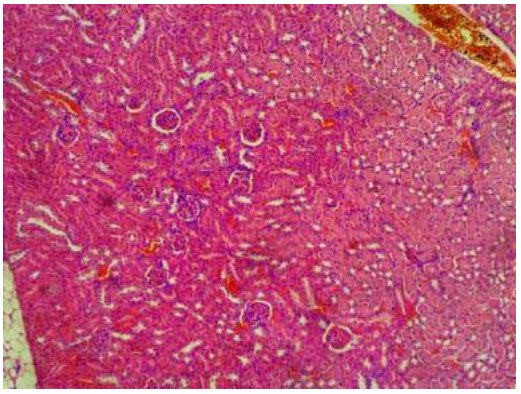


viii. 5000mg/kg (vascular congestion)

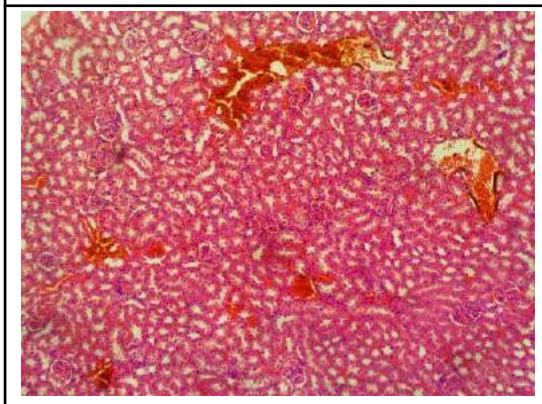
Figure 2: Histologic sections of liver excised from animals that received only distilled water (control) and those that received 300-5000mg/kg of aqueous extract of P*icralima nitida*. The liver tissue show parallel radially arranged plates of hepatocytes with the portal space and periportal zone filled with a smooth to slightly floccular pink fluid material common with edema and congested aggregates of red blood cells were also seen in both control and treated group



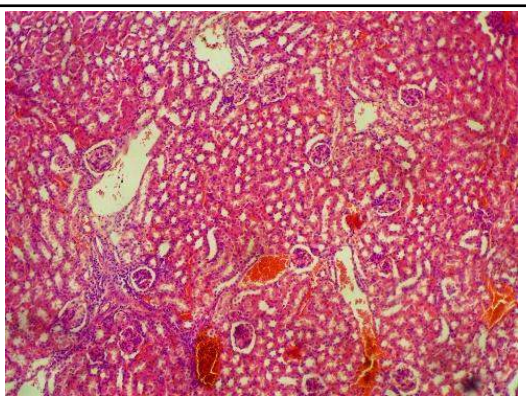
ix. Control (vascular congestion)



x. 300mg/kg (mild vascular congestion)

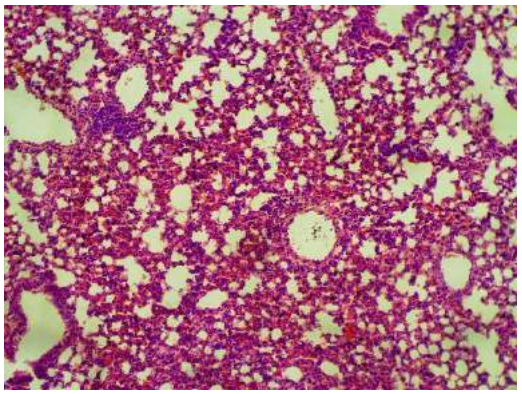


xi. 2000mg/kg (vascular congestion)

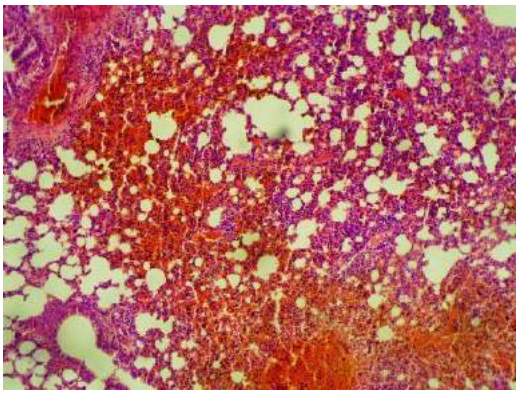


xii. 5000mg/kg (vascular congestion)

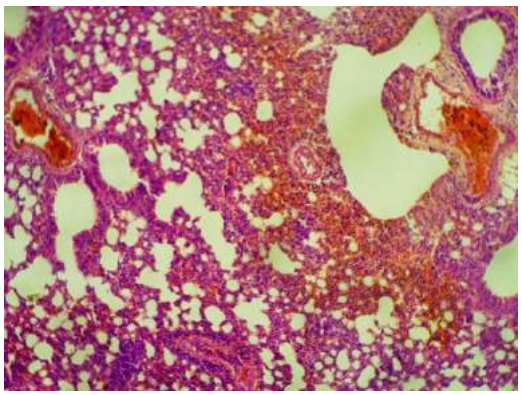
Figure 3: Histologic sections of kidney excised from animals that received only distilled water (control) and those that received 300-5000mg/kg of aqueous extract of P*icralima nitida*. Kidney tissue show normocellular glomerular tufts disposed on a background containing viable tubules. Congested blood vessels were seen



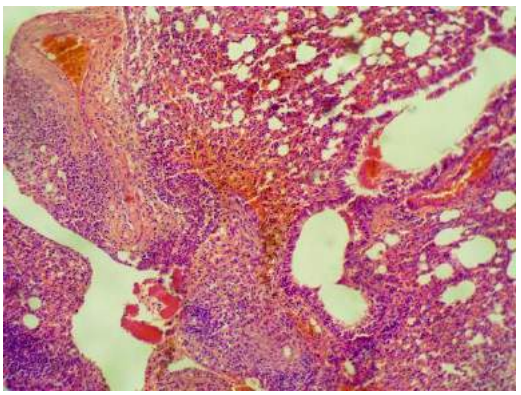
xiii. Control (vascular congestion)



xiv. 300mg/kg (lung inflammation)



xv. 2000mg/kg (lung inflammation)



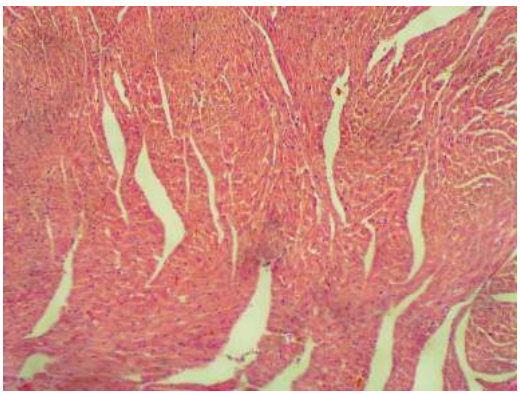
xvi. 5000mg/kg (lung inflammation)

Figure 4: Histologic sections of lungs excised from animals that received only distilled water (control) and those that received 300-5000mg/kg of aqueous extract of P*icralima nitida*. Lung tissue show some alveolar filled air spaces, the vessels in the walls of the alveoli are distended and the capillaries are congested with aggregates of many red blood cells

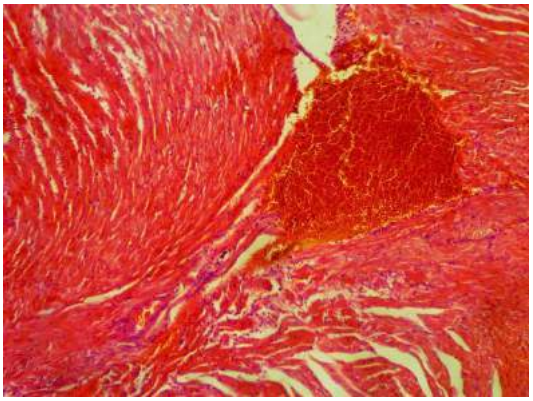
3.4 Histopathological Effect of Picralima nitida 80% Ethanolic extracts

The histologic structures of the vital organs (kidney, liver, lungs and heart) of the control group (untreated animals) and treated groups are shown in figures 5 to 8.

The histologic sections of the heart, liver, kidney, and lungs of the animals that received mid dose (2000mg/kg), and high dose (5000mg/kg) of 80% ethanolic extract of P*icralima nitida* had some histopathological changes. Similar injuries were also seen in the untreated group (control) thus the cause of the histopathology is unclear.

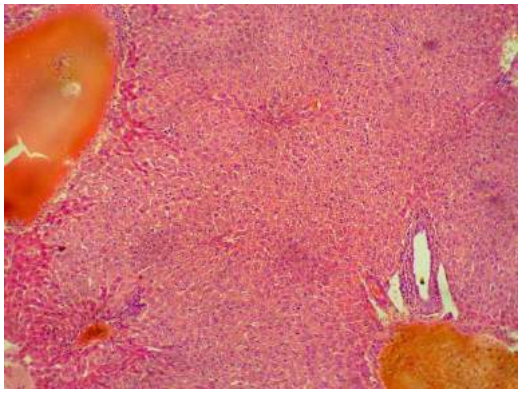


a. Control (normal)

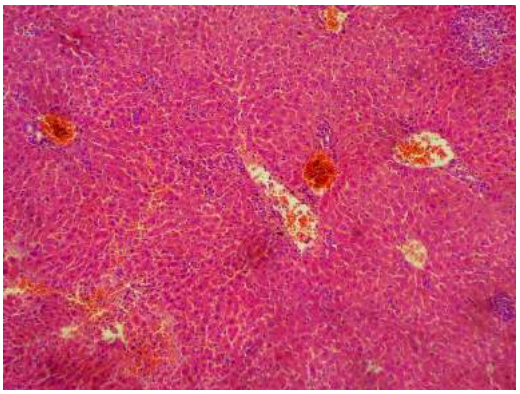


b. 2000mg/kg (vascular congestion)

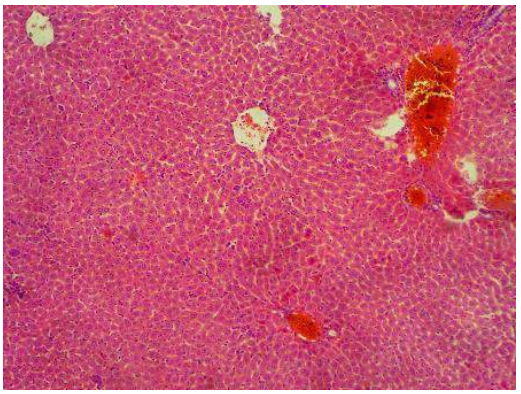
Figure 5: Histologic sections of heart show interlacing fascicles of cardiac myocytes/ myocardial cells. The heart sections for untreated and those treated with 300-5000mg/kg of ethanolic extract of P*icralima nitida* were normal



c. Control (vascular congestion)

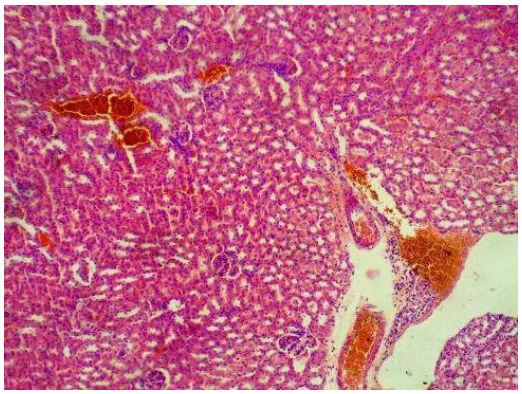


d. 2000mg/kg (mild vascular congestion)

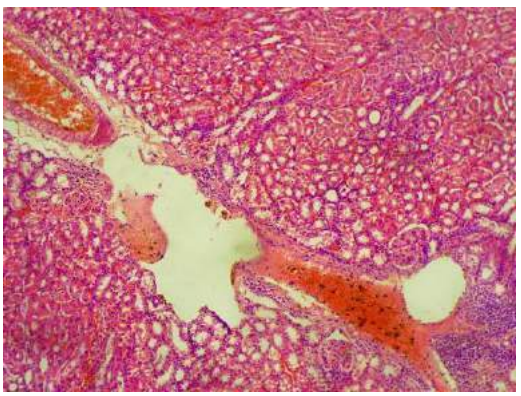


e. 5000mg/kg (mild vascular congestion)

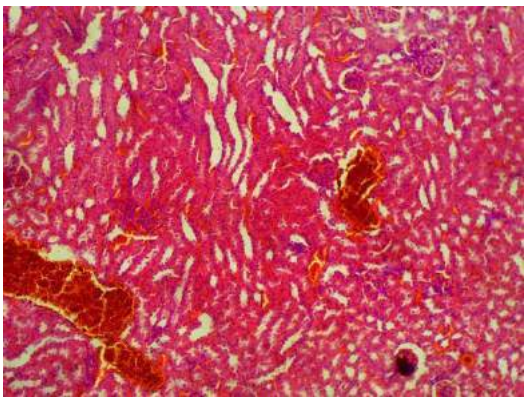
Figure 6: Histologic sections of liver tissue show parallel radially arranged plates of hepatocytes with the portal space and periportal zone filled with a smooth to slightly floccular pink fluid material common with edema and congested aggregates of red blood cells also seen. The liver sections for untreated and those treated with 300-5000mg/kg of ethanolic extract of P*icralima nitida* had mild to severe vascular congestion



f. Control (vascular congestion)

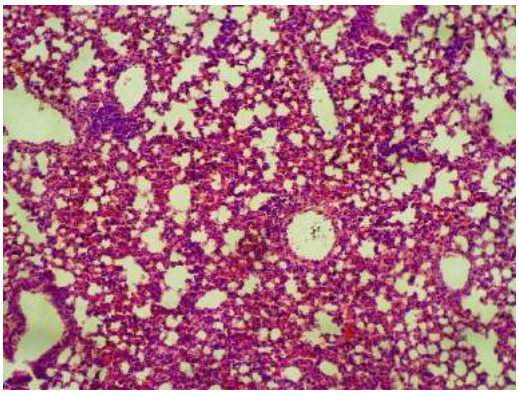


g. 2000mg/kg (vascular congestion)

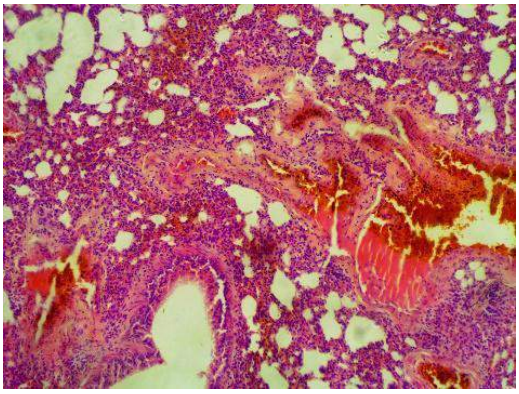


h. 5000mg/kg (vascular congestion)

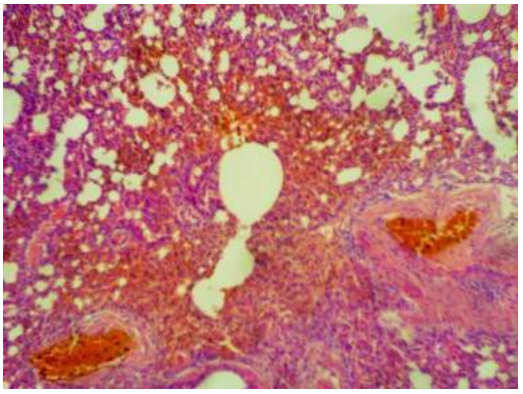
Figure 7: Histologic sections of kidney tissue show normocellular glomerular tufts disposed on a background containing viable tubules. Congested blood vessels are seen. The kidney sections for untreated and those treated with 300-5000mg/kg of ethanolic extract of P*icralima nitida* had mild to severe vascular congestion



i. Control (vascular congestion)



j. 2000mg/kg (vascular congestion)



k. 5000mg/kg (vascular congestion)

Figure 8: Histologic sections of lung tissue showing some alveolar filled air spaces, the vessels in the walls of the alveoli are distended and the capillaries are congested with aggregates of many red blood cells. The lung sections for untreated and those treated with 300-5000mg/kg of ethanolic extract of P*icralima nitida* had mild to severe vascular congestion

IV. DISCUSSION

The phytochemical analysis showed the presence of alkaloids, tannins, phenols, saponin, flavonoids, and terpenoids which indicated that these plants contain important plant antioxidants and the plant may thus have free radical scavenging activities. Our result is similar with previous research by Nkere and Iroegbu who reported the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, and steroids in P*icralima nitida* [11]. The LD_{50} value obtained from our acute toxicity study indicates that P*icralima nitida* is safe. Our results of the LD_{50} (≤2000mg/kg) is similar to the value reported by Koffi *et al* who estimated the LD_{50} values for the acute oral and intraperitoneal toxicity studies for P*icralima nitida* to be 3000 mg/kg [12].

The absence of histological alterations in the heart histology of both untreated and treated groups at low (300mg/kg) to the highest dose (5000mg/kg) indicate that aqueous extract is safe on the heart even at the highest dose. However, the presence of some histologic changes in the liver, kidneys, lungs of the untreated (control) and treated groups that received aqueous extract of P*icralima nitida* indicate toxic adverse effects which may not be induced by the P*icralima nitida* because the same histological injuries were also seen in the control. Hence, the cause of the adverse effects is unknown.

The histologic sections of the 80% ethanolic extract of P*icralima nitida* treated animals showed some histopathology in the heart, liver, kidney, and lungs of the animals that received mid

dose (2000mg/kg), and high dose (5000mg/kg). This could be a spontaneous occurrence because similar injuries were also seen in the untreated group (control) thus the cause of the histopathology is unclear.

V. CONCLUSION

The phytochemical screening revealed that P*icralima nitida* contains important antioxidants and other phytochemicals with various health benefits. While the acute toxicity assessment of the aqueous and 80% ethanolic extracts of Picralima *nitida* indicate that P*icralima nitida* is safe for oral consumption and subsequent therapeutic activities.

Conflict of Interests: The authors declare no conflict of interest.

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