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Acute Toxicity of Methanol Extract of *Picalima nitida* in Swiss Albino Rats

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ABSTRACT

The acute toxicity of root methanol extract of Picalima nitida was studied in Swiss albino rats. The rats were randomly distributed into four groups of three animals each. The groups were respectively administered 0, 10, 100 and 1000mg/kg body weight methanol extract of Picalima nitida intra peritoneally in a single dose and monitored frequently for 24h and daily for 13 days in the first phase of the experiment. In the second phase of the experiment, the animals were administered single doses of the extract at 0, 200, 400, 800 and 1600mg/kg body weight (bw) of the extract intraperitoneally and monitored frequently for 24h and 13 days respectively. The number of deaths in a group was recorded. The results of the second phase experiment were used to calculate the LD50 of the plant extract. All surviving animals were sacrificed after 14 days. Selected organs of the animals i.e. liver and kidney of both the dead and sacrificed animals were removed and stored in 10% formal saline ready for histopathological analysis.

Tissue specimens of the organs were examined histopathologically after processing and staining with haematoxylin and eosin. The results of the biochemical parameters indicated elevations. Lesions were observed in the liver and kidney of rats administered 800, 1000 and 1600mg/kg of methanol extract of Picralima nitida. From this result, the LD50 was calculated to be 557 mg/kg. The results indicate that the extract may be toxic at high doses.

Key words: Picralima Nitida, Methanol Extract, Acute Toxicity, Swiss Albino Rats and Histopathology.

INTRODUCTION

Plants have formed the basis of traditional system of medicine that have been in existence for thousands of years and continue to provide human kind with medical remedies (Gurib Frank, 2006). They are also central to people's livelihood. Indigenous people living in a particular area depend on the plant parts to fulfill their needs and often have considerable knowledge of their uses (Evans, 2002). Plants have always been among the common sources of medicines, either processed as traditional preparations or used to extract pure active principles. The bioactive ingredients that have the therapeutic activity in plants used in traditional practice are mostly unidentified and traditional healers believe in the holistic nature of their treatment, while many research groups screen plant extracts for new promising therapeutic candidates for infectious diseases (Valdes et al., 2008). Since most plants have medicinal properties, it is of most important that their efficacy and toxicity risks are evaluated (Sofowora, 1993). Traditional medicinal plants are an important element of indigenous medical system in the world (Kong et al., 2003). Traditional system of medicine is one of the centuries-old practice and long serving companion of human kind in the fight against diseases and in leading a healthy life. Indigenous people have been using the unique approach of their traditional system of medicine for centuries and among the most renowned are the Chinese, Indian, African systems of medicine (Karunamoorthi et al., 2012). They offer an accessible and affordable health care regime and serve as an important source of livelihood for indigenous rural populations. *Picralima nitida* is a rich source of alkaloids belonging to the family Apocynaceae (common name; Akuamma plant, Igbo; Osi-Igwe). Many herbalists have claimed to use the leaves, roots, seeds or stembark for treatment of various fevers, hypertension, jaundice, gastrointestinal disorders and malaria (Iwu, 1993 and Etukudo, 2003). This plant also has a very high anti-malarial activity (Duwiejua et al., 2002). Two third of the world's population mainly in the developing countries relies entirely on medicinal plants as their primary form of health care. The medicinal values of the plant are believed to be due to the presence of bioactive compounds such as glycoside, saponin, tannin, flavonids, terpenoid, alkaloids etc (Leven et al., 1979). Several plant materials by instincts were used to combat various disease conditions (Lambo, 1979) even without the medicinal effect of this plant is being considered. With advances in western scientific methods, most of these so-called' medicinally important plants came under chemical scrutiny and were tested for their ability to exhibit the professed healing effects both in vivo and in vitro before they could be certified to have protective effects (Sofowara, 1982). There should be clear understanding of

therapeutic value of such plant and their toxicity (Summer 2000). As such, a scientific approach needs to be applied towards the use of plant extracts in managing aliments. However, the safety of *P. nitida* is important in relation to its therapeutic actions. This study is aimed at determining the possible acute toxicity of methanol extract of *P. nitida* in Swiss albino rats.

MATERIALS AND METHODS

Plant Material and Extract Preparation

The plant *Picalima nitida* was collected in Umukabia, Ehime Mbano, Imo State, Nigeria, and identified by a Taxonomist at the Herbarium Section of the Department of Biological Sciences, Micheal Opara University, Umudike, Abia State. The Voucher number of the plant is 29271. The whole plants of *Picalima nitida* used for this study were collected in May. The plants were dried under the shade in the laboratory for two weeks and ground into powdered form using laboratory mortar. Extraction of this powdered form of the plant was carried out with methanol for (4hx2) using Soxhlet apparatus. The extract was stored in the refrigerator at -4ºC until required.

ANIMALS

The male Swiss albino rats used for this study were purchased from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu State Nigeria. The animals were about eleven weeks old and weighed between 95-100g initially.

Acute Toxicity Evaluation/Determination of Median Lethal Dose LD50

The method of Lorke (1983) was used to study the acute toxicity of methanol extract of *Picralima nitida*. For the first phase of treatment, the doses of 10mg/kg, 100mg/kg and 1000mg/kg of methanol extract of *P. nitida* were administered intraperitoneally to the test animals in each treatment group respectively (i.e., 10, 100 and 1000mg/kg groups). Three mice were used for each of the test/treatment groups and control. The control groups were administered normal saline which was used in dissolving the extract. The animals were observed continuously for the first 1h and then every 30 min during the first 24h for the onset of any immediate toxic signs and daily during the 13 days observation period to record any delayed acute effects. The number of deaths in a group was also recorded. For the second phase of treatment, the doses of 200mg/kg, 400mg/kg, 800mg/kg and 1600mg/kg of methanol extract of *P. nitida* were equally administered intraperitoneally to the test animals in each treatment group respectively (i.e., 200, 400, 800 and 1600mg/kg groups). This second phase doses were chosen based on the results of the first phase of treatment. The animals were observed as previously described and the number of deaths in a group was equally recorded. The results of the second phase experiment were used to calculate the LD50 of the plant extract.

The LD50 was calculated according to the method outlined by Lorke [1983] as: Geometric mean of the doses for which there was no mortality x the next dose for which mortality occurred = x mg/kg. All surviving animals were sacrificed after 14 days. Selected organs of the dead or sacrificed animals (liver and kidney) were removed and stored in 10% formal saline solution ready for histopathological analysis. Detailed histopathological study of the animals' organs administered with the methanol extract of *P. nitida* was carried out.

Histopathological Analysis of Organs

Tissue specimens of the organs were examined histopathologically after processing and staining with haematoxylin

and eosin. The method of Okoro (2002) was used in the processing, staining and embedding of organs.

RESULTS

Acute Toxicity Evaluation of Chloroform Extract of *Picralima nitida*

In the first phase of treatment, mice administered the methanol extract of *P. nitida* developed clinical signs of toxicity (loss of appetite, loss of agility, convulsion and loss of vision) after 40mins of the post treatment period with 1000mg/kg of the extract. Two rats in this 1000 mg/kg group died after 1h of treatment. There were no clinical signs of toxicity observed with the rat in the 100mg/kg, 10mg/kg and the control groups either, immediately or during the post treatment period. Also no mortality occurred in these groups (Table 1). In the second phase of the experiment, rats administered the extract developed clinical signs of toxicity (loss of appetite, loss of agility, convulsion and loss of vision) after 20 mins of the post treatment period with 1600 mg/kg of the extract. Three of the mice in this 1600mg/kg group died after 28 mins of treatment. The only clinical sign of toxicity observed in the 800mg/kg group was loss of agility and appetite after 2h of treatment and one rat died in the same group after 2h 20mins. No clinical signs of toxicity were observed with the rats in the 200mg/kg, 400mg/kg and control groups. No mortality occurred in these groups during the 14-day observation period (Table 1). From this result, the LD50 of the methanol extract of *P. nitida* was calculated to be 557mg/kg (Table 1). Body weight loss was observed in the 1600 mg/kg and 800 mg/kg treated groups compared to the normal control and the other treatment groups.

Table 1. Acute Toxicity Studies (LD_{50}) of methanol extract of *P. nitida*

			Proportion of Death		
Plant	**Experiment**	**Dose (Mg/Kg)**	**Within 24 hrs**	**After 24 hrs**	**After 2 weeks**
P. nitida (Root methanol extract)	Phase 1	10	0/3	0/3	0/3
		100	0/3	0/3	0/3
		1000	2/3	2/3	2/3
	Phase 2	200	0/3	0/3	0/3
		400	0/3	0/3	0/3
		800	0/3	1/3	1/3
		1600	3/3	3/3	3/3

LD_{50} of *Picralima nitida* = 557mg/kg

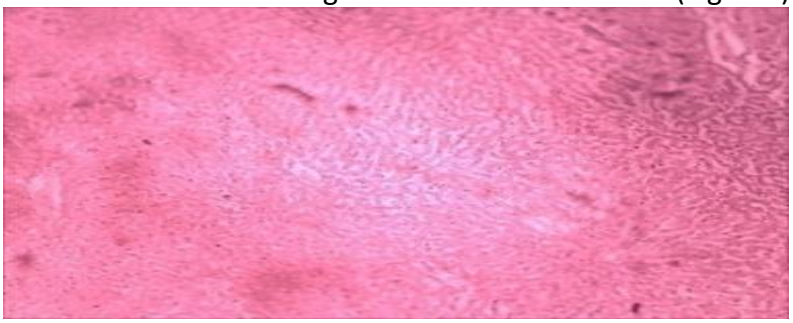
The extract produced significant changes ($p<0.05$) in the average weight of the liver and kidney of the rats treated with 800 mg/kg, 1000mg/kg and 1600 mg/kg of methanol extract of *P. nitida* compared to the other treated groups and untreated controls (Tables 2).

Table 2. Average weight of organs of rats for acute toxicity studies of *P. nitida*

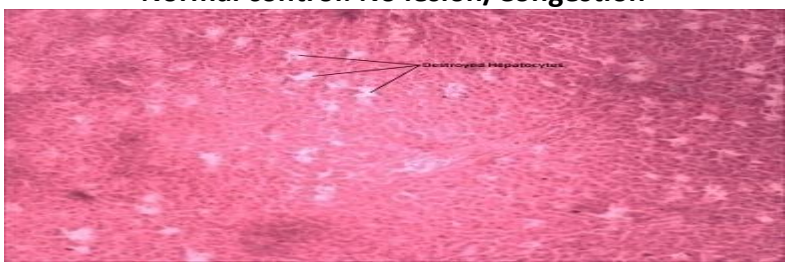
S/N	Doses (mg/kg)	N	Average weight of organs	
			Kidney	Liver
1	Control	3	3.50 ± 0.01^{e}	7.11 ± 0.01^{g}
2	10	3	3.51±0.01	7.11±0.01
3	100	3	3.51±0.01	7.21±0.02
4	200	3	3.52±0.01	7.25±0.03
5	400	3	3.54±0.01	7.32±0.03
6	800	3	3.71 ± 0.03^{f}	7.99 ± 0.07^{h}
7	1000	3	3.75 ± 0.05^{f}	8.08 ± 0.08^{h}
8	1600	3	3.77 ± 0.15^{f}	8.38 ± 0.11^{h}

N = Number of animals in a group

Macro and microscopic observations indicated congestion/ destroyed hepatocytes in the liver of the animals treated with 800 mg/kg, 1000 mg/kg and 1600 mg/kg of methanol extract of *P. nitida* (Fig. 1). There were destroyed corpuscles observed in the kidney of the treated animals with 800 mg/kg, 1000 mg/kg and 1600 mg/kg of the extract (Fig. 2).
There were no lesions observed in the organs of the normal control (Fig. 1-2).

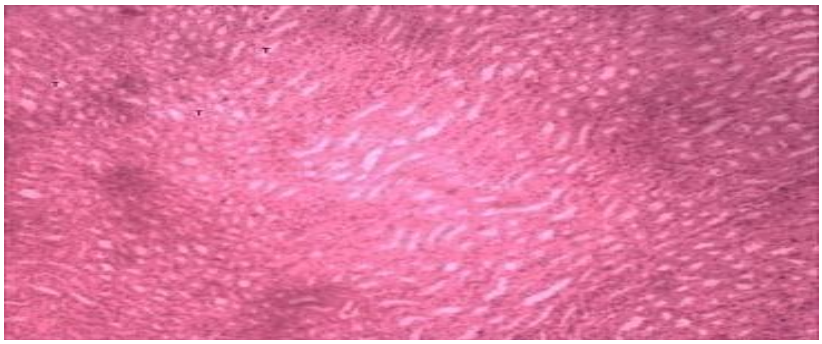


Normal control: No lesion/Congestion

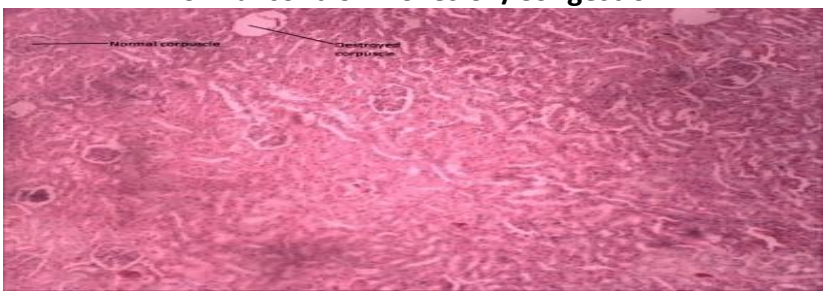


Destroyed hepatocytes (N)

Fig. 1. Histopathology of liver of rat following intraperitonial acute administration of methanol extract of *P. nitida* (x 400).



Normal control: No lesion/Congestion



Destroyed corpuscles (C)

Fig. 2. Histopathology of kidney of rats following intraperitoneal acute administration of methanol extract of *P. nitida* (x 400).

DISCUSSION

The toxicological evaluation of any plant extract helps in assessing the efficacy of the extract at any particular dose (Bacchi 1996). In this study, liver and kidney damage were experimentally induced by the administration of a single dose of 800mg/kg, 1000mg/kg and 1600mg/kg body weight of methanol root extract of *P. nitida* to animals. Some factors are cable of affecting the toxicity of medicinal plant either during extract preparation or environmental condition. Determination of the LD50 was used as a standard measure for assessing the toxicity of the extracts which was 557mg/kg and reveals no obvious sign of toxicity. However, administration of methanol root extract of *P. nitida* above the LD50 resulted into a significant ($p< 0.05$) elevation in the average weight of the liver and kidney of the rats treated with 800, 1000, 1600 mg/kg, demonstrating a sign of toxicity on the experimental animals. Ethanol extract of *P. nitida* in Swiss albino rats, at higher doses has earlier been reported (Francois et al., 1996) to produce significant elevations in total plasma protein which indicates that the liver function was adversely affected by the extract. This is also in agreement with the report of Fakeye et al (2004). However, the alkaloid extract of *P. nitida* stem bark also produce inflammation and necrosis of liver hepatocytes accompanied by reduction in neutrophilic count and a corresponding increase in lymphocytic. Moreover, when there is damage on the hepatocytes and corpuscles, some biochemical parameters can be affected causing an increase in them when compared with the control like in serum protein level. It is possible that the hyper protein anemia observed in studies may also be the consequence upon the damaged kidney and liver (**Andrew and Goslin, 1999**). This is in agreement with the report of Tijani et al (Andrew and Goslin, 1999). In that study, it was reported that the ethanolic extract shows significant increase in the serum protein level in rats. Also Ene et al. (2014) showed that the test plant extract was modestly toxic to the experimental animal. This is in agreement with the methodology and results of this present study.

CONCLUSION

A single intra peritoneal dose of 800 mg/kg, 1000 mg/kg and 1600 mg/kg of methanol extract of *P. nitida* (root) was able to induce mortality or toxic effects in rat while lower doses did not induce mortality. This shows that this extract is toxic at higher doses but safe at lower doses.

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