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Research Paper

Safety Evaluation of Ethanol Leaf Extract of *Picralima nitida* Stapf (Apocynaceae)

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Abstract

The safety of the ethanol leaf extract of P. nitida, a popular Nigerian traditional antidiabetic remedy was investigated. For the acute toxicity study, 1000 – 5000 mg/kg of the ethanol leaf extract were administered to albino mice and obvious toxic symptoms and mortality 24 h post administration of the extract were determined. The median lethal dose (LD_{50}) of the extract was determined. In the subchronic study, 750-3000 mg/kg of the extract were administered daily for 90 days. The food and water consumption, body weight changes, as well as heamatological and biochemical parameters were determined periodically. The phytochemical constituents of the extract were also investigated. Phytochemical analysis revealed the presence of alkaloids, saponins and tannins.The estimated LD_{50} was upto 5000 mg/kg. There was no reduction in body weight and non- significant ($p > 0.05$) alteration in Hb concentration, PCV and RBC count. However, there were significant ($p < 0.05$) elevation of WBC count, ALT, AST and ALP at the 61st and 91st days of the study. Hepatic photomicrograph reveals liver cell degeneration and rupture of the hepatocytes at high doses. At lower dose (750 mg/kg) no significant ($p > 0.05$) change was recorded for all the tested parameters. This study suggests that the leaf extract of P. nitidais safe at low doses but may be toxic when used chronically at higher doses.

Key words: P. nitida, toxicity, biochemical and heamatological parameters

INTRODUCTION:

Popular observations on the use and efficacy of medicinal plants has led to their frequent prescription even when their chemical constituents are not completely known [1]. The decreasing efficacy of synthetic drugs, cost, accessibility and increasing contradiction of their usage make the usage of natural drugs topical [2].

There is a growing awareness by scientific and medicinal communities of the importance of medicinal plants in the health care system of many developing countries [3].Traditionally, it is believed that natural products are safe [4,5]. This assumption to a large extent has influenced the indiscriminate use of these formulations by many particularly among the rural populace [6]. The safety of herbal medicines is of particular importance because the majority of these products is self-prescribed and is used to treat minor and often chronic conditions [7]; most

patients taking these preparations are not aware of their potential adverse effects. Though bioactive compounds derived from medicinal plants can be useful therapeutically, they can have serious dose-related side effects [8]. In the south Eastern Nigeria, *P. nitida* leaf is widely used in traditional medicine for the treatment of diabetes but little is known about its safety. Since diabetes is a chronic disease, this study evaluated the acute and chronic toxicities of the aqueous ethanol extract of *P. nitida.*

MATERIAL AND METHOD

Plant material

The leaves of *P. nitida* were collected from Agulu, Anambra State, Nigeria and authenticated by a taxonomist, Mr. PaulinusUgwuozor of Botany Department NnamdiAzikiwe University, Awka, Anambra State, Nigeria. The leaves were subsequently cleaned, air-dried and pulverized.

Phytochemical test

The phytochemical analysis of the leave extract were carried out using standard methods of Odebiyi and Sofowora [9], Treas and Evans, [10].

Plant Extraction

A 2kg quantity of the powdered plant material was extracted with 10L of 70% ethanol using maceration technique for 7days. The extract was filtered and the filtrate concentrated in vaccuo at 40^0C using the rotary evaporator and stored inside the refrigerator at 4^0C till further use.

Animals

Swiss albino rats of both sex(150 $\pm$ 20g) were employed for the study. The animals were obtained from the animal house of the Department of Pharmacology and Toxicology, NnamdiAzikiwe University, Agulu Campus, Nigeria. The animals were housed in standard laboratory conditions and fed with rodent feed (Guinea feed Nigeria Ltd). They were allowed free access to food and water*ad libitum*.

Acute toxicity study:

This was done using Lorke method [11]. A total of 13 mice were used for the study. The study was done in two phases. In the first phase, three groups of three animals each were given 1000, 2000 and 4000mg/kg of the extract (p.o). The mice were observed for signs and symptoms of toxicity and mortality over a period of 24 h. Four mice were administered 5000mg/kg of the extract in the second phase and observed for another 24 h, post administration.

Sub-chronic toxicological studies:

Sixty rats were randomly divided into four groups of fifteen rats per group. The extract was administered orally at doses 750, 1500, 3000 mg/kg to the test groups once daily for 90 days. The control group received 10ml/kg of normal saline. Physical observation of the animals were done daily. The rats were weighed every 7 days.

Five rats from each group were anaesthetized using chloroform on the 31^{st}, 61^{st} and 91^{st} days of the study. Blood samples were collected through retro-orbital puncture and was used for the

estimation of haemoglobin (Hb), packed cell volume(PCV), red blood cell (RBC) and white blood cell count (WBC) [12].

For the estimation of the serum enzyme levels, the blood samples were allowed to coagulate for 30 minutes and the clear serum was separated by centrifuging at 2500 rpm for 10 minutes and was then used for analysis of biochemical hepatic markers- Aspartate aminotransferase (ALT) [13], Alanine aminotransferase (ALT) [13] and alanine phosphatase (ALP) [14]. Histopathological studies were done on liver isolates from different dose groups of the animals and at various times.

Statistical analysis:

The results were analysed using SPSS version 16 and presented as mean $\pm$ SEM. The significance between control and extract treated group were determined using students t-test and one way ANOVA. p value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION:

The hydro-ethanol extract of P. nitida reveals some useful phytocompounds(Table 1) which may have contributed to the toxicity exhibited by this extract. Alkaloids and saponins and their derivatives from some plants have been documented to have hepatotoxic effect [15-17]. For the acute toxicity studies, no death was recorded for all the animals that received 1000, 2000, 4000 and 5000 mg/kg of the extracts. No sign of weakness or anorexia was observed within 24 hours of administration, an indication of acute safety of the extract [18]. There was no reduction in body weights of the animals (Table 2) an indication that the extract does not affect the feeding activities of the animals. This was supported by the fact that there was no observed reduction in the physical activity of the animals. Assessment of haematological parameters can be use to determine the extent of deleterious effect of foreign compounds including plant extracts on blood [19]. Non-significant ($p>0.05$) elevation of hemoglobin concentration, packed cell volume and red blood cell count (Tables 3, 4 and 5, respectively) is an indication that the extract is unlikely to cause anemia on chronic use [12].The significant ($p<0.05$) elevation of white blood cell (WBC) count (Table 6) at higher doses on the 61st and 91st days was probably due to normal response to foreign bodies.Biochemical studies showed a dose- and time-dependent significant ($p<0.05$) elevation of serum Aspartate aminotransferase (AST), Alanine aminotransaminase (ALT), and serum Alkaline phosphatase (ALP) (Tables 7, 8 and 9 respectively). The liver associated enzymes, ALT, AST and ALP are indirect measures of liver homeostasis. Hepatocellular injury leading to permeability of intracellular enzymes into the bloodstream is accompanied by elevated ALT and AST [20,21] while ALP elevation is associated with cholestasis due to biliary obstruction or hepatic infiltration.Histopathological examination of the liver reveals liver cell degeneration and rupture of the hepatocytes (Fig 1-5).

Table 1: Phytochemical analysis of the plant extract

Phytocompounds	**Degree of occurrence**
Flavonoids	+
Alkaloids	+++
Tannins	++
Saponins	+++
Steroids	+
Fats and oil	+
Carbohydrates	+

Key: + Mild present, ++ Moderately present, +++ Strongly present

Table 2: Effect of extract on body weight

Mean Body weight (g)			
Treatment (mg/kg)	31^{ist} day	61st day	91st day
Control	13.46 $\pm$ 3.0	18.49 $\pm$ 3.1	21.30 $\pm$ 3.5
750	15.45 $\pm$ 3.6	25.49 $\pm$ 2.9	27.40 $\pm$ 3.6
1500	12.80 $\pm$ 4.2	17.80 $\pm$ 2.6	20.50 $\pm$ 3.0
3000	16.76 $\pm$ 3.3	24.76 $\pm$ 3.4	26.10 $\pm$ 2.9

Dose of extract in mg/kg n/gp = 5

Table 3: Effect of extract on hemoglobin concentration (Hb)

Mean Hemoglobin concentration (g/dl)				
Treatment(mg/kg)	Pre-treatment	31st day	61st day	91st day
Control	12.5± 1.2	12.8±1.0	13.0±1.5	13.6 ± 1.4
750	12.4± 0.8	12.6±1.4	12.9±1.2	13.6± 1.4
1500	12.0 ± 1.1	12.9±1.1	13.3±1.0	13.9± 1.3
3000	12.5 ± 1.2	13.2±1.3	13.5±1.1	14.1± 1.5

Dose of extract in mg/kg n/gp = 5

Table 4: Effect of extract on packed cell volume (PCV)

Mean Packed cell volume (%)				
Treatment(mg/kg)	Pre-treatment	31st day	61st day	91st day
Control	35.7± 2.2	37.8± 2.0	38.4± 1.5	40.3± 1.2
750	36.3± 1.8	37.3± 1.3	38.2± 1.2	38.8± 1.5
1500	35.0± 1.2	36.9± 1.0	39.3± 1.4	40.2± 1.1
3000	36.5± 1.5	38.2± 1.2	39.5± 1.0	40.5± 1.0

Dose of extract in mg/kg n/gp = 5

Table 5: Effect of extract on red blood cell

Mean red blood cell (10^{12}/L)				
Treatment(mg/kg)	Pre-treatment	31st day	61st day	91st day
Control	4.7± 1.9	5.2± 1.5	5.6± 1.5	6.2±1.4
750	4.5± 2.0	5.0 ± 1.4	5.6± 1.2	6.4±1.6
1500	4.9± 1.8	5.5± 1.6	5.8± 1.0	6.5±1.2
3000	4.3± 1.6	5.8± 1.2	5.9± 1.1	6.8± 1.6

Dose of extract in mg/kg n/gp = 5

Table 6: Effect of extract on white blood cell

Mean white blood cell (x10^{9}/L)				
Treatment(mg/kg)	Pre-treatment	31st day	61st day	91st day
Control	5.3± 1.5	5.6± 1.3	5.9± 1.2	6.4± 1.5
750	5.2± 1.6	5.8± 1.3	6.5± 1.1	6.8± 1.4
1500	5.4± 1.2	5.5± 1.6	*6.8± 1.4	*7.3± 1.3
3000	5.6± 1.4	5.8± 1.1	*7.0± 1.0	*8.0±1.2

Dose of extract in mg/kg n/gp = 5 *p<0.05 compared with control

Table 7: Effect of extract on serum Aspartate aminotransferase (AST)

Mean serum Aspartate aminotransfrase (U/L)				
Treatment(mg/kg)	Pre-treatment	31st day	61st day	91st day
Control	48.2± 1.7	55.6± 1.4	57.6± 1.5	61.8 ±1.2
750	48.6± 1.2	50.5± 1.4	56.4± 1.3	64.6± 1.1
1500	46.9± 1.5	50.2± 1.3	*68.6± 1.2	*75.8±0.9
3000	47.5± 1.4	53.4± 1.2	*76.5± 1.3	*82.4±1.0

Dose of extract in mg/kg n/gp = 5 *p<0.05 compared with control

Table8: Effect of extract on serum Alanine aminotransferase (ALT)

Mean serum Alanine aminotransferase (U/L)				
Treatment(mg/kg)	Pre-treatment	31st day	61st day	91st day
Control	36.4± 1.5	38.8± 1.7	43.3± 1.5	48.3± 1.5
750	36.2± 1.4	37.6± 1.6	42.5± 1.3	48.4± 1.2
1500	36.7± 1.7	45.3± 1.5	*58.5± 1.3	*66.2 ± 1.0
3000	35.3± 1.3	*53.6± 1.5	*62.4± 1.2	*69.1± 1.4

Dose of extract in mg/kg n/gp = 5 *p<0.05 compared with control

Table 9: Effect of extract on Alkaline Phosphatase (ALP)

Mean serum Alanine phosphate (U/L)				
Treatment(mg/kg)	Pre-treatment	31st day	61st day	91st day
Control	65.3± 1.5	69.3± 1.2	75.4± 1.0	79.6± 1.5
750	68.3± 1.4	70.6± 1.3	77.5± 1.6	80.2± 1.1
1500	66.5± 1.6	68.5± 1.5	81.4± 1.4	*88.6± 1.6
3000	67.8± 1.1	73.3± 1.7	*85.4 ± 1.3	*92.4±1.3

Dose of extract in mg/kg n/gp = 5 *p<0.05 compared with control

Figure 1: Photomicrograph of the liver that served as control.

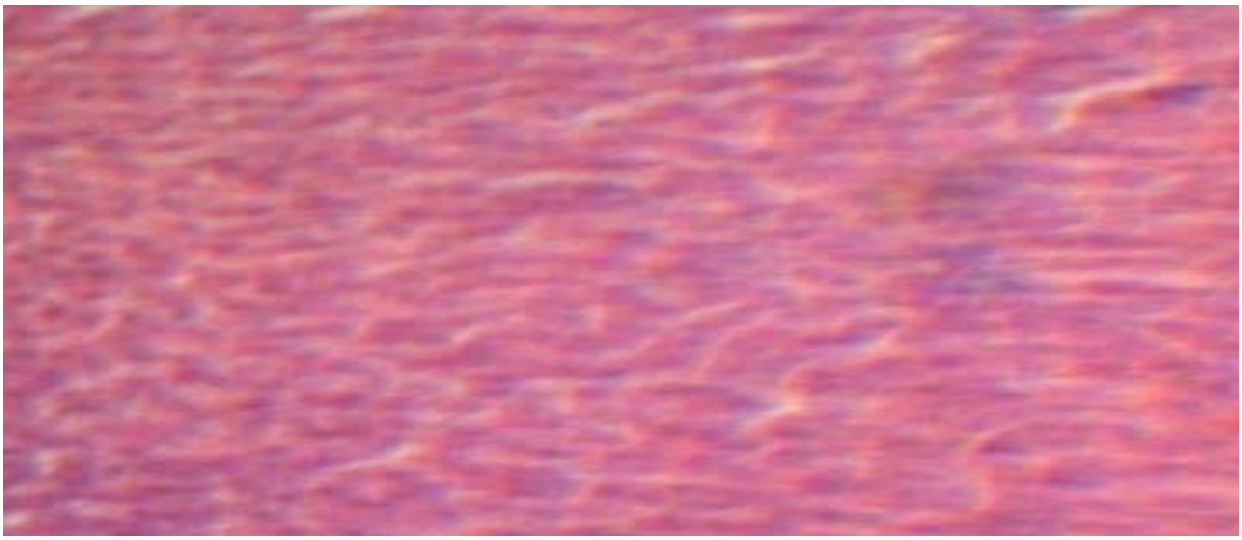


Figure 2: Photomicrograph of the liver at dose 750mg/kg for the 1nd month showing no damage to the liver.

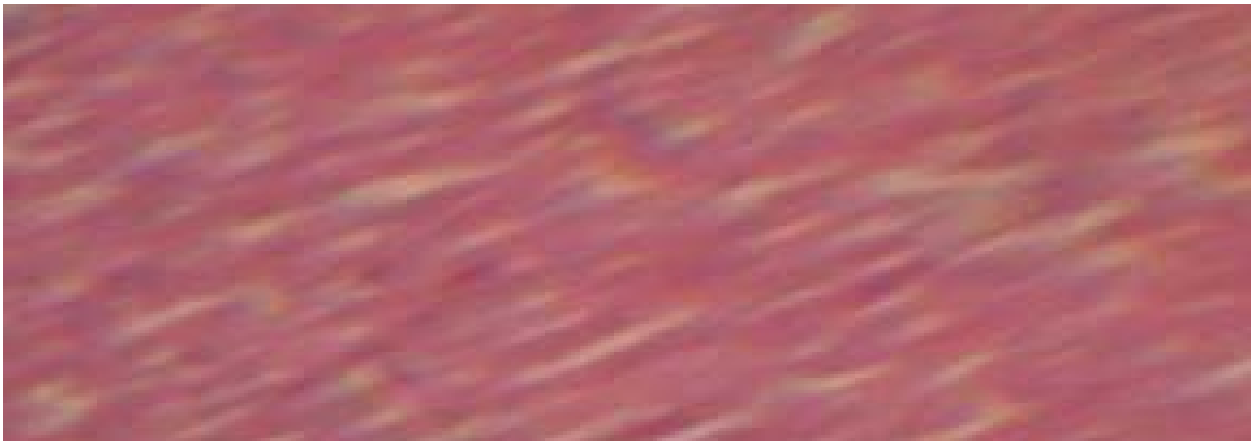


Figure 3: photomicrograph of the liver at dose 3000mg/kg for the 1st month showing no damage to the liver

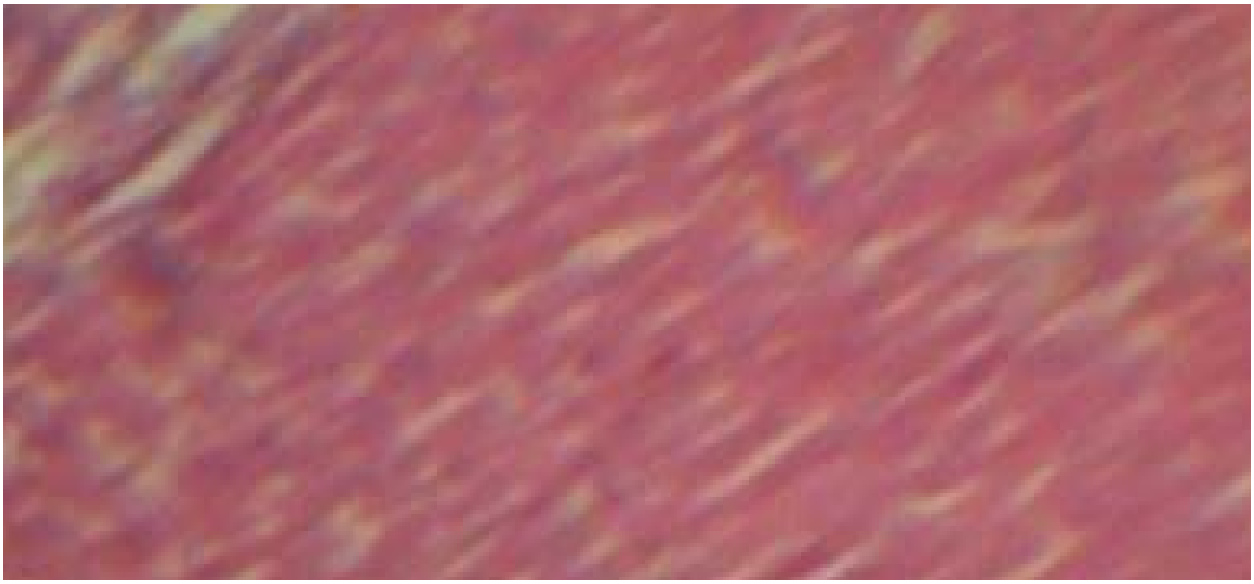


Figure 4: Photomicrograph of the liver at dose 1500mg/kg for the 3rd month showing damage to the liver

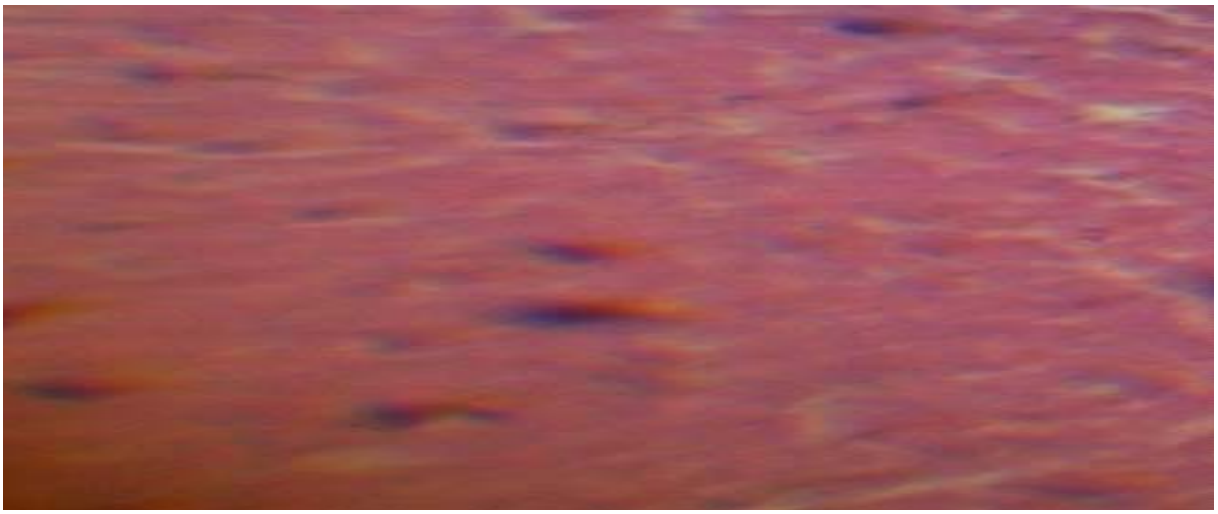
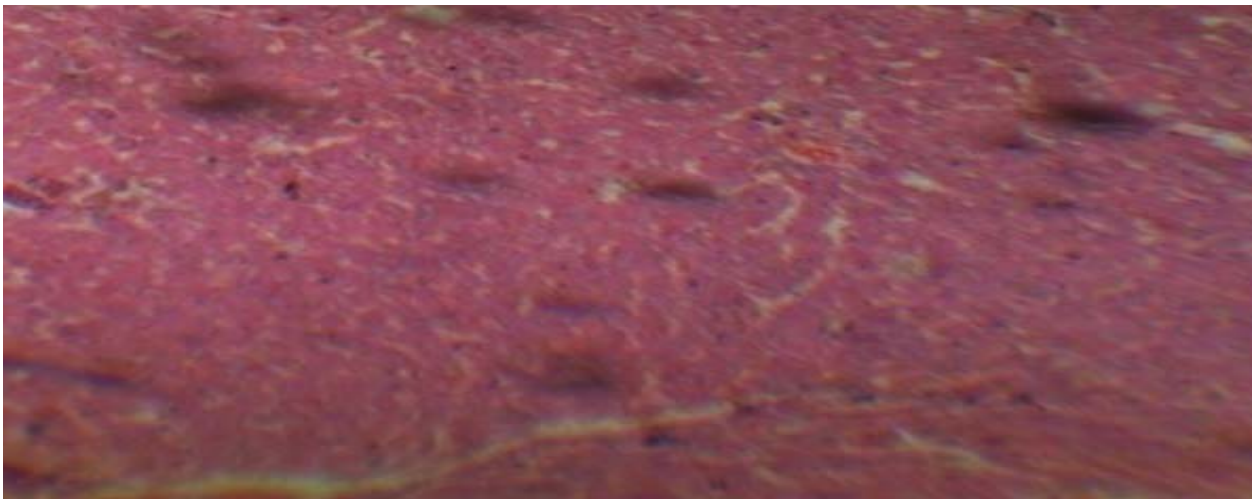


Figure 5: photomicrograph of the liver at dose 3000mg/kg for the 3rd month showing hepatocellular rupture



CONCLUSION

These results provide evidence for the safety profile of ethanol leaf extract of *P. nitida*, supporting its chronic use only at low doses in the folkloric treatment of diabetes.

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