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# Hepatoprotective potentials of *Picralima nitida* against *in vivo* carbon tetrachloride-mediated hepatotoxicity

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## ABSTRACT

This research aimed at investigating the *in vivo* Carbon tetrachloride ($CCl_4$)-mediated hepatotoxicity of methanolic seed extract of *Picralima nitida* (*P. nitida*) using Wistar rats. Twenty five (25) rats randomly selected into five groups of five animals were used in this research. Group 1 was administered Normal saline (Negative control); Group II was administered 1 ml of Carbon tetrachloride only (Positive control/ Reference drug); Group III, IV and V got 10 ml *P. nitida* extract + 1ml Carbon tetrachloride; 100 ml *P. nitida* extract + 1ml Carbon tetrachloride and 1000 ml *P. nitida* extract + 1ml Carbon tetrachloride respectively. Results show that treatment with *P. nitida* extract had no adverse effect on the body weight of Wistar rats. Biochemical analysis show increase in CAT and GSH which are good antioxidant agents. Photomicrographs show moderate amelioration from steatosis caused by Carbon tetrachloride in the treatment groups. Further study is recommended to verify if *P. nitida* seed extract can completely ameliorate and possibly reverse fat degeneration of liver cells induced by Carbon tetrachloride.

**Keywords:** *Picralima nitida*, Hepatotoxicity, $CCl_4$.

## INTRODUCTION

According to the World Health Organization, about 80% of the world's population depends wholly or partly on plant- derived pharmaceuticals [1]. This is because the exorbitant cost and associated intolerable side-effects of most conventional pharmaceuticals prevent most people from being able to acquire them [2]. A wide range of medicinal plants is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include leaves, stems, flowers, fruits, twigs exudates and modified plant organs. While some of these raw plants parts are collected in smaller quantities by the local communities and traded in the market as the raw material for many herbal industries [3]. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.

*Picralima nitida* (Stapf) T. Durand & H Durand (family: Apocynaceae) is a West African glabrous tree commonly known as Abeere (in Yoruba, Southwest Nigeria) [4]. *P. nitida* is a small glabrous tree of about 15–22 m in height with a dense evergreen crown found in West and Central Africa [5]. In Nigeria, it is found in the rain forest zone of the southern part of the country with the local names, Osu (Edo) and nkpokiri (Ibo) [6]. In African folk medicine, *Picralima nitida* plant has wide therapeutic applications including the local treatment of pain, swellings, infections, gastric ulcers, liver diseases, diabetes mellitus, obesity and management of labour at term [1, 5-7]. The aqueous seed extract of the plant has also been reported to be relatively safe when administered orally [8]. *P. nitida* in new studies has shown great ability to lower cholesterol within one day. It lowers blood sugar levels thus helping those with diabetes [8]. Some of these previous investigations revealed that it is highly valued in the local management of hypertension, sexually transmitted infections, and as immune booster [7].

Hepatic damage is a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [9]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. Therefore, it is necessary to search for alternatives to liver disease treatment in order to replace currently used drugs with those of doubtful efficacy and safety [10]. However, limited data are available regarding the liver toxicity of *P. nitida*. Even, the findings of the available research works are controversial. However, there is a report indicating that *P. nitida* has no liver toxicity effect in experimental animals [11]. This controversy indicates that there is need for further investigation in this light. On the other hand, there is a fact that the liver is a major organ for metabolism of foreign substances and also functionally interposed between the site of absorption and the systemic circulation. These conditions render the liver not only the most important organ for detoxification of foreign substances but also a major target of their toxicity [11]. This study is aimed at evaluating *P. nitida*

methanolic seed extract for *in vivo* hepatoprotective effect, oxidative stress reduction in comparison with that of a standard drug 'Carbon Tetrachloride' $CCl_4$ and histopathological studies of the plant extract.

## MATERIALS AND METHODS

### Collection of plant Materials

The ripe fruits of *P. nitida* were collected from Egor Local Government Area of Edo State in Nigeria. It was first identified by Professor Macdonald Idu of Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The fruits were further authenticated by Forest Research Institute of Nigeria, Ibadan, where a specimen with Voucher number FHI107618 was deposited.

### Preparation of plant extract

The fresh fruits of *P. nitida* were cut open and the pulp removed. The seeds were squeezed out of the pulp and dried in the sun for a week. Dried seeds were reduced to powder using a mechanical grinder. 500 g of the dry seeds were ground to powder using a mechanical blender. Thirty grams of the fine powdered sample was de-fatted with petroleum ether for 24 hours and was extracted with methanol for 48 hours in a 1 liter Pyrex beaker and was left to stand for 72 h. After 48 h, the homogenate was rigorously shaken intermittently for 6 hours and was rapidly filtered through a piece of clean muslin cloth. The filtrate was then transferred to an aerated oven preset at 40 °C for drying until a deep brown solid residue was obtained. The weight of the solid residue was 23 g, giving a yield of 76.67% ($^{w}/_{w}$). The extract was scooped and stored in an air-moisture-tight container which was kept in a refrigerator maintained at −4 °C until ready for use.

### Experimental animals

Adult albino Rats (8–14 weeks old) used in this study was got from the Animal House of the National Vertinary Research Institute (NVRI) Vom Jos, Plateau State, Nigeria, after ethical approval was obtained. The animals were maintained on standard feed (Ladokun Feeds, Ibadan, Nigeria) rat-pellets and water which were made available *ad libitum*. The animals were maintained at temperature between 28–30 °C, humidity of 55 ± 5%, and photoperiod of approximately 12 hour of light (06:30 h – 18:30 h) alternating with approximately 12 hour of darkness (18:30 h – 06:30 h).

Drugs and chemicals: The carbon tetrachloride used in this research was manufactured by May and Baker Ltd, Dagenham, England. Teco Diagnostic kits (Lakeview Ave. Anaheim, USA) were used for the analysis of biochemical parameters. All the other chemicals were of analytical grades.

### Experimental Design for Hepatoprotective Activity

Twenty five rats were randomly distributed into five groups of five each. The hepatoprotective activity of plant extracts was tested using the $CCl_4$ model. Carbon tetrachloride hepatotoxicity was induced in rats according to the method described by [12]. Group I rats served as the untreated control and were orally administered with of normal saline while group II was administered $CCl_4$. Animals in Groups III–V were orally administered 10, 100, 1000 mg/kg of the methanolic seed extract of *Picralima nitida* + 1 ml $CCl_4$, respectively. The animals were closely monitored for behavioral and general signs of toxicity such as feeding and drinking pattern, restlessness, and mortality, etc. within the first 24 hrs. Surviving mice were further observed for 8 days for delayed toxicities or death. Ethical consent was sought before this research was undertaken.

### Assessment of Hepatoprotective Activity

In the present study, the hepatoprotective activity of *picralima nitida* was estimated biochemically and histopathologically. After a week administration/treatment, the animals were dissected under 2% chloroform anesthesia. Blood from each rat was withdrawn by cardiac puncture into non-heparinized tubes, allowed to clot for 30 minutes at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 min. The separated sera were used for the estimation of some biochemical parameters; AST, ALT, ALP, total bilirubin and total protein.

For histopathological studies, liver from animals in each group was removed after dissection and preserved in Bouin fluid (picric acid+formalin+acetic acid). Then, representative blocks of liver tissue from each lobe were taken and processed for paraffin embedding, using the standard microtechnique described by Galighor and Kozloff [13]. Section (5 µm) of liver stained with hematoxylin and eosin was observed microscopically and photographed (Olypus, CS21) for histopathological studies.

### Statistical Analysis:

The results are expressed as Mean ± S.E.M. The significant differences at ($P$<0.05) among Means were analyzed using one way ANOVA. Where, 'n' represents the number of animals in each experimental group.

## RESULT

**Effect of methanolic seed extract of *P. nitida* on mean body weights:** The extract had no significant effect on the body weight of the rats but rats pre-treated with 10mg/kg (Group III) of the extract showed loss in body weight as shown on Table 1.

**Table 1:** Effect of pre-treatment of rats with methanolic seed extract of *P. nitida* against $CCl_4$ induced hepatotoxicity on mean body weights

| Groups | Initial body weight | Final Body Weight | Body Weight Difference | % Weight Change |
|---|---|---|---|---|
| I | 269.51±3.44 | 295.51±3.47 | 26.00±0.03 | 8.80 |
| II | 365.01±4.31 | 370.60±5.19 | 5.59±4.31 | 1.51 |
| III | 352.03±1.08 | 347.36±7.37 | 4.67±6.29 | 1.34 |
| IV | 311.09±5.42 | 318.32±5.44 | 7.23±0.02 | 2.27 |
| V | 283.34±8.23 | 295.42±7.44 | 12.08±0.79 | 4.09 |

**Key:** Group I- Normal saline; Group II- 1 ml $CCl_4$ only; Group III-10 mg/kg extract + $CCl_4$; Group IV-100 mg/ kg extract + $CCl_4$; Group V-1000mg/kg extract + $CCl_4$. n=5 animals per group.

The result of *P. nitida* methanolic seed extract on $CCl_4$ induced hepatotoxicity in rats presented in Tables 2 and 3. The result shows that there are no significant differences ($P$<0.05) in some biochemical parameters- AST, ALT, ALP, DB,TB, TP, CAT and GSH tested when compared with normal saline- control. In groups 3 and 4, treatment showed that CCl4 effect was significantly reversed on the enzymes.

Group 5 showed a significant increase in ALP ($P$<0.05) when compared to $CCl_4$.

**Table 2:** Effect of pre-treatment of Albino Rats with methanolic seed extract of *P. nitida* against $CCl_4$ Induced hepatotoxicity on some biochemical parameters

| Group | Drug Treatment | ALT U/l | AST U/l | ALP U/l | TP Mg/dl | TB Mg/dl | DB Mg/dl |
|---|---|---|---|---|---|---|---|
| **1** | Vehicle control | $8.20^{a}$±0.85 | $16.60^{a}$±0.68 | $108.53^{b}$± 9.32 | $5.42^{b}$±0.39 | $0.4^{a}$±0.11 | $0.39^{a}$±0.11 |
| **2** | $CCl_4$ | $9.07^{a}$±0.94 | $12.47^{a}$±5.59 | $93.87^{b}$±4.21 | $5.55^{b}$±0.16 | $0.43^{a}$±0.07 | $0.29^{a}$±0.04 |
| **3** | 10 mg/kg of *Pn* + $CCl_4$ | $8.76^{a}$±0.56 | $16.80^{a}$±2.66 | $93.83^{ab}$ ±9.95 | $4.95^{ab}$±0.26 | $0.41^{a}$±0.07 | $0.35^{a}$±0.86 |
| **4** | 100 mg/kg of *Pn* + $CCl_4$ | $8.87^{a}$±1.50 | $13.87^{a}$±0.59 | $73.57^{a}$±9.95 | $4.74^{ab}$±0.22 | 0.53±0.43a | $0.32^{a}$±0.80 |
| **5** | 1000 mg/kg of *Pn* + $CCl_4$ | $6.03^{a}$±0.78 | $11.00^{a}$±0.51 | $115.90^{b}$ ±5.73 | $5.13^{a}$±0.46 | $0.42^{a}$±0.72 | $0.36^{a}$±0.09 |

Values are expressed as Mean ± SEM; NS: Not significant; *: Significant at ($P$<0.05). n=5.

**Table 3:** Effects of *P. nitida* seed extract on some biochemical parameters on $CCl_4$ induced hepatotoxicity in Rats

| Groups | GSH | MD×$10^6$ | SOD | CAT | Liver weight (g) |
|---|---|---|---|---|---|
| I | $135.00^{a}$±14.85 | $3.53^{a}$±0.90 | $1.20^{a}$±0.00 | $147.95^{a}$±12.06 | $5.86^{a}$±0.62 |
| II | $148.91^{a}$±14.83 | $2.14^{ab}$±0.25 | $1.47^{a}$±0.27 | $143.86^{a}$±3.91 | $6.40^{a}$±1.12 |
| III | $156.42^{a}$±8.17 | $1.83^{ab}$±0.26 | $0.67^{a}$±0.27 | $160.46^{a}$±9.24 | $7.04^{a}$±0.49 |
| IV | $138.33^{a}$±19.60 | $2.90^{ab}$±0.57 | $1.20^{a}$±0.00 | $159.60^{a}$±15.32 | $6.75^{a}$±1.29 |
| V | $176.26^{a}$±7.59 | $4.02^{b}$±0.74 | $0.93^{a}$±0.70 | $191.77^{a}$±22.60 | $7.94^{a}$±2.04 |

Values are Mean ± SEM; n=5.

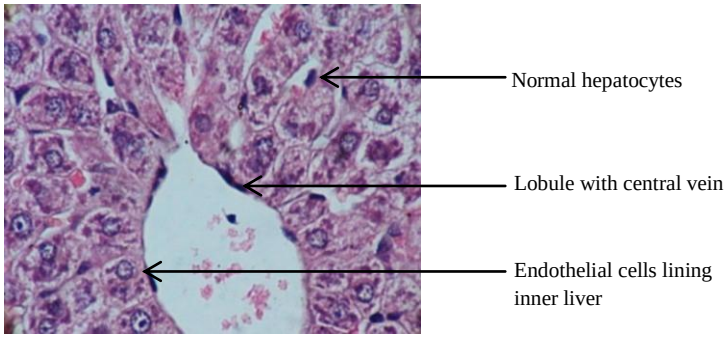


**Plate 1:** Histopathology of Rat liver- Group I: control group fed with normal saline. H&E 400x.

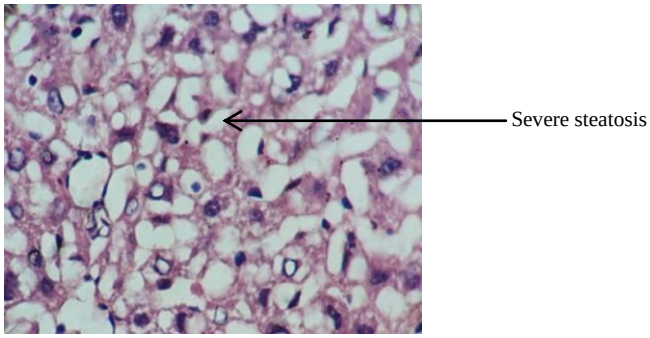


**Plate 2:** Histopathology of Rat liver- Group II: 1ml of $CCl_4$ only, **C**, severe fatty degeneration; Steatosis evident by 'empty cells' and peripheral nuclei. H&E 400x.

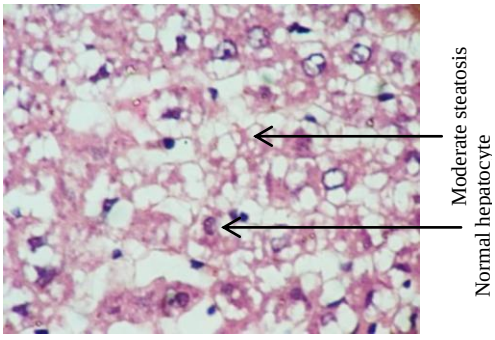


**Plate 3:** Histopathology of Rat liver- Group III: 10 ml *P. nitida* seed extract +1ml CCl4. moderate fatty degeneration and regeneration of normal hepatocytes. H&E 400x.

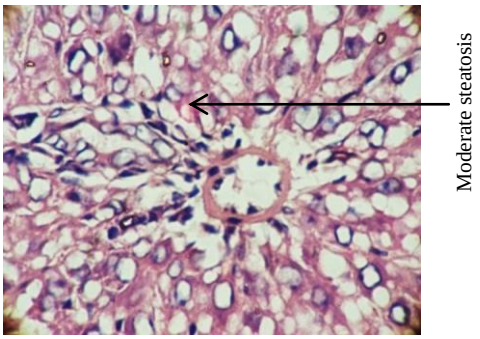


**Plate 4:** Histopathology of Rat liver- Group IV: 1 ml CCl4 and 100 ml of *P. nitida* seed extract; moderate fatty degeneration with presence of inflammatory cells, nuclei of hepatocytes. H& E 400x.

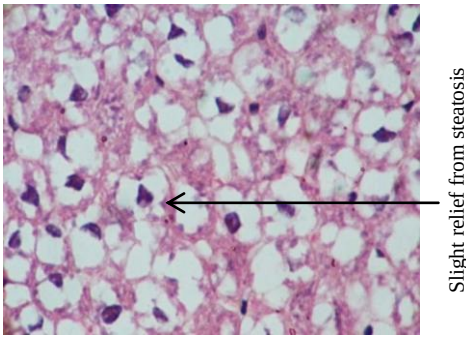


**Plate 5:** Histopathology of Rat liver-1 ml $CCl_4$ and 1000 ml of *P. nitida* seed extract; fatty regeneration evident by the replacement of hepatocytes with empty spaces and peripheral nuclei. Cell nuclei of some hepatocytes are seen in some at the center points. H& E 400x.

## DISCUSSION

Liver damage induced by carbon tetrachloride ($CCl_4$) is a commonly used model for the screening of hepatoprotective drugs [14]. When rats are treated with $CCl_4$, it induces hepatotoxicity by metabolic activation. $CCl_4$ selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical ($CCl_4$) which combined with cellular lipid sand proteins in the presence of oxygen to induce lipid peroxidation [14]. CAT and GSH are useful and reliable markers of antioxidant status while MDA is a sensitive and reliable marker for lipid peroxidation [15]. This study demonstrates that a single dose of 1 ml $CCl_4$ injection produced elevated levels of CAT and GSH (Table 3). An increase in total bilirubin andtotal protein (Table 2) was also found, which corroborates with the research findings of Etuk *et al*., [16]. Increased CAT activity observed in aqueous stem bark extracts of *B. aegyptiaca* treated rats is indicative of the ability of the plant extract to protect against lipid peroxidation/oxidative damage arising from acetaminophen intoxication [12, 17]. Table 1 shows that treatment with *Picralima nitida* methanolic seed extract induced no significant weight loss on Wistar rats. Photomicrographs from this study, show that *Picralima nitida* has some anti-hepatotoxic activities as it helps to ameliorate steatosis from severe to moderate level in the treatment doses used in this study (Plates 1-5). *Picralima nitida* from the findings in this study possesses the capacity to reverse the damage caused by $CCl_4$ on liver cells. Steatosis; toxic damage of liver cells represented by fat deposition in the cytoplasm of cells were gradually cleared off and replaced with normal hepatocytes after treatment with *P. nitida* extract.

## CONCLUSION

*P. nitida* shows slight hepatoprotective activity in this study. However, further research is required to further ascertain if or not an increase in treatment doses of *P. nitida* can completely ameliorate steatosis in liver cells of Wistar Rats poisoned with $CCl_4$.

**Conflicts of interest**
There are no conflicts of interest.

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