The Pharmaceutical and Chemical Journal, 2017, 4(2):66-71

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Phytochemical screening and anti- hepatotoxic effect of ethanolic root extract of *Terminalia macroptera* (guill & perr) on induced liver injury Wister rats

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Abstract This paper reports the anti-hepatotoxic effect of the ethanol extract of the root of *Terminalia macroptera (Guill and Perr)* on carbon tetrachloride induced liver injury Wister rats. The animals were separated into five groups (A, B, C, D & E) consisting of three animals per group. The rats in group A were induced subcutaneously with 100mg/kg body weight and monitored for liver injury after 48 hours while groups C, D & E were treated with 210, 450 and 640 mg/kg of the extract intraperitoneally after inducement with carbon tetrachloride in addition to normal feed. High dose of the ethanol root extract of *Terminalia macroptera (Guill and Perr*) significantly reduced ($p< 0.05$) hepatitis in Wister rats. Preliminary qualitative phytochemical screening of ethanol root extract from this experiment showed the presence of alkaloids, flavonoids, resins, saponins, steroids, glycosides

Keywords Phytochemical Screening, *Terminalia macroptera* (Guill and Perr), anti-hepatotoxicity, carbon tetrachloride

Introduction

There have been steady and gradual interest in recent times over herbal therapy and scientific screening of plants; crude or purified. Literature dates of some of the plant based activities on antimicrobial supports this claims [1-4]. Especially in developing countries, these interests may not be farfetched from the fact that, there is high cost of orthodox medicine, increased drug resistant cases and low therapeutic index of synthetic drugs [5-6]. It has been seen that the action of plant active effect for prevention and therapy for diseases through self induce medication, which is one way in the selection of herbal for medication use heath services safety.

Plants extracts should not only be effective on diseases but also safe in terms of consumption. Therefore the relationship of plant extract with its action on microorganism should also be accompanied with its effect on toxic possibility [7].

Terminalia macroptera (Guill & perr) a plant wide spread gregarious savanna tree which may be readily recognized by the prominent tuft of nearly stalkless pale green leaves, and by its large fruits. The tree is about 13m high and 2m in girth, with an open spreading crown. In Guinea-Bissau, West-Africa, it is used by traditional healers for the treatment of hepatitis and venereal diseases [8]. The traditional uses of this species corroborate the findings of several authors that *T. macroptera* possesses strong *in vitro* antibacterial and antifungal effects. Root extracts of *T. macroptera* have been reported to give slight activity against *Candida albicans* and interesting profile of activity against enteropathogenic microorganisms, including *Shigella dysenteriae*, with ethanol and water extracts being especially active [9]. This study reports the anti-hepatoxicity effect of ethanol roots extract of Wister rats

Materials and Methods

15 Wister albino rats grouped into five of three in each group were purchased from the Department of Biological Sciences Bayero University, Kano State, Nigeria. The animals were weighed, housed and kept in standard condition in the animal house of the department. The animals were fed with commercial feeds and were given normal water.

Fresh root *T. macroptera guill & perr* were obtained in February, 2011 from Idah in kagi State, Nigeria, and was authenticated by Mallam Musa Gallah of the herbarium unit of Ahmadu Bello University Zaria, Kaduna state Nigeria.

Extract Preparation

The roof of *T. macroptera guill and perr* was air dried and pulverized into fine powder. The powder (500 g) was percolated with 2.5 L ethanol at room temperature for two weeks, and then filtered. The filtrate was concentrated using rotavapour machine at 40 °C. The crude ethanol extract obtained (45 g) was kept in a refrigerator until use.

Phytochemical Screening

The ethanol fraction (3.0 g) of the root of *T. macroptera* guill and perr was subjected to preliminary phytochemical screening, to identify the secondary metabolites present.

LD_{50} test

This was carried out in two phases, comprising a total of twelve rats in the first phase and three rats in the second phase. At each phase, the extract was dissolved in normal saline and administered intraperitoneally. The animals were observed for two weeks for any clinical signs.

Phase 1: Rats were grouped into four of three each. Group 1, 2 and 3 were administered 10, 100 and 100 mg/kg body weight of extracts respectively.

Phase 2: Rats were grouped into three, one rat per group. They were given 1600, 2900 and 5000 mg/kg body weight extract to deter mine the value of the LD_{50}.[10].

Carbon tetrachloride induced liver damage

Carbon tetrachloride (CCl_4) solution administered was prepared by dissolving 1ml of CCl_4 in pure vegetable oil (which was used as solvent) and making up the volume to 50 cm^3 *i.e.* 2 % w/v. The volume of CCl_4 administered was determined based on the weight of the laboratory rats using the relation:

$$\text{Volume (ml)} = \frac{50cm^3 \times \text{Dose(mg)} \times \text{weight of rats}}{1000g \times 1590mg}$$

Where 1590 mg is the weight per ml of CCl_4

Experimental Design of Wister Rats Distribution

The rats were randomly assigned into five groups of three rats (n=3) each. Group A, C, D and E were induced with 100mg/kg body weight of carbon tetrachloride and group B was given normal food and saline water. Group C, D and E were treated with 630 mg/kg, 450 mg/kg and 210 mg/kg body weight ethanol root and root- bark extract after two days of inducement of carbon tetrachloride respectively.

Liver Function Test

Animals were sacrificed according to W.H.O standards and serum samples were taken to Aminu Kano Teaching hospital, Kano State, Nigeria for liver function investigation: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP).

Statistical Analysis

The ALT, AST and ALP values were presented in the form of mean ± SD. Values in all groups were compared with negative control (group B) using the analysis of variance (ANOVA). For all analyses the level of statistical significance was fixed at $p<0.05$[7]

Results and Discussion

Table 1: LD_{50} RESULT

Group	Dose (mg/kg bw)	Rats/ experiment	Number of death recorded after 24 hrs
Phase-1	10	3	Nil
	1,00	3	Nil
	1,000	3	Nil
Control	0	3	Nil
	1,600	1	Nil
Phase 2	2,900	1	Nil
	5,000	1	Nil

Table 2: Statistical analyses of results of rat's induced with CCl_4

Group	ALT	AST	ALP
A	54 ± 6.08^a	86.3 ± 3.51^a	25.67 ± 1.15^a
B	41.3±2.31	68±6	26±2

Key= Mean ± Standard Deviation, $^aP > 0.05$ (significant change) $^bP < 0.05$ (no significant change)

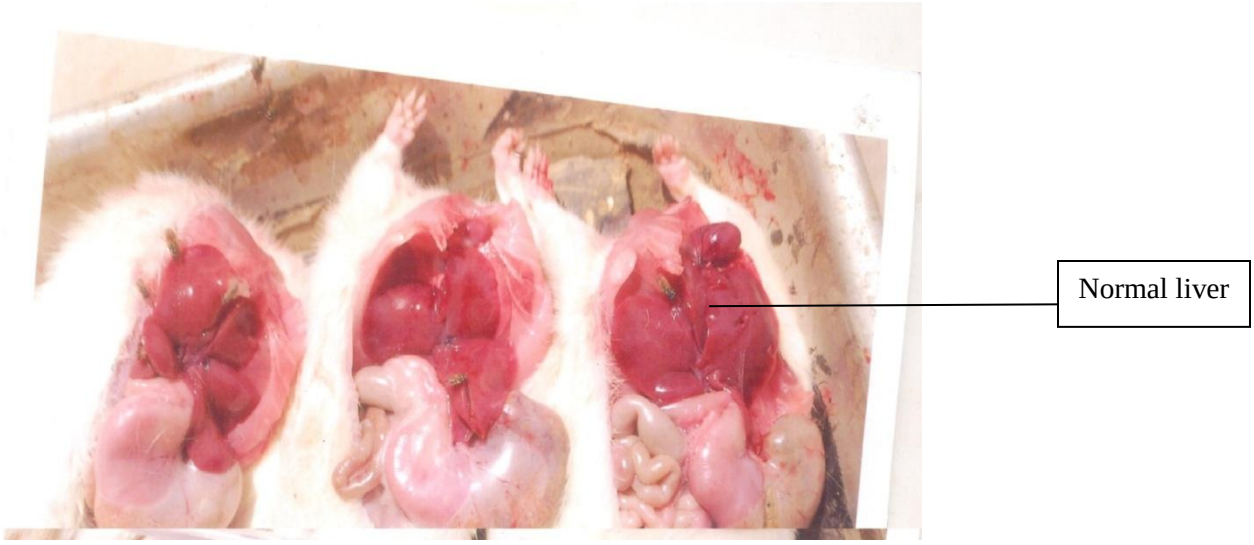

Figure 1: Liver of rat that were fed normal feed & water

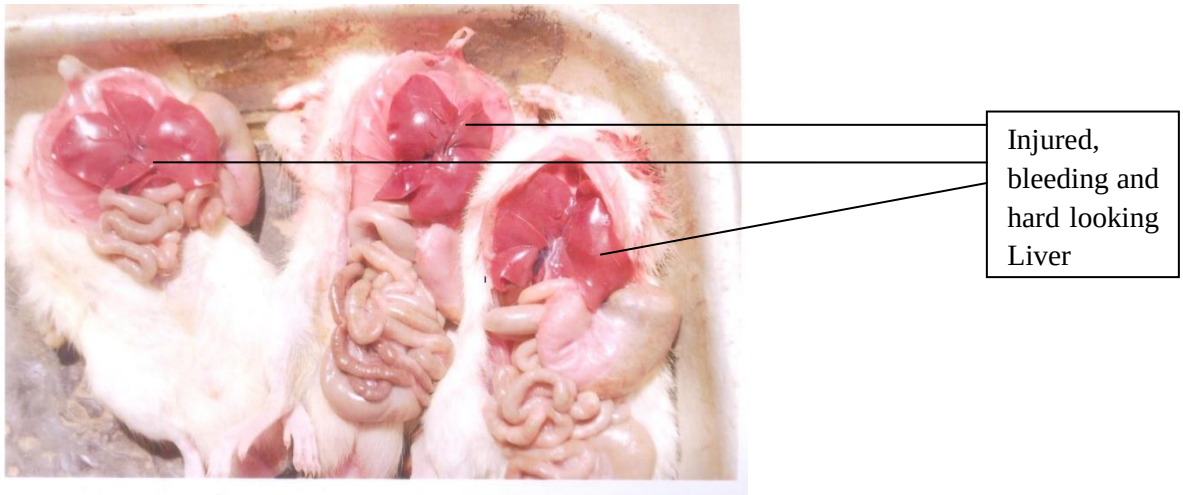

Figure 2: Liver of rats induced with 100mg/kg CCl_4

Table 3: Analyzed results of rats treated with roots and root-bark extract

Group	ALT	AST	ALP
C	37.3 ± 3.05^{b}	72.7 ± 8.08^{b}	27.3 ± 0.57^{b}
D	64.3 ± 4.04^{a}	91 ± 3.46^{a}	26.7 ± 1.53^{a}
E	42.3 ± 8.14^{a}	87.3 ± 7.37^{a}	27.3 ± 2.08^{a}

Key= Mean ± Standard Deviation, $^{a}P > 0.05$ (significant change) $^{b}P < 0.05$ (no significant change)

Figure 3: Liver of rats treated with 640mg/kg extract

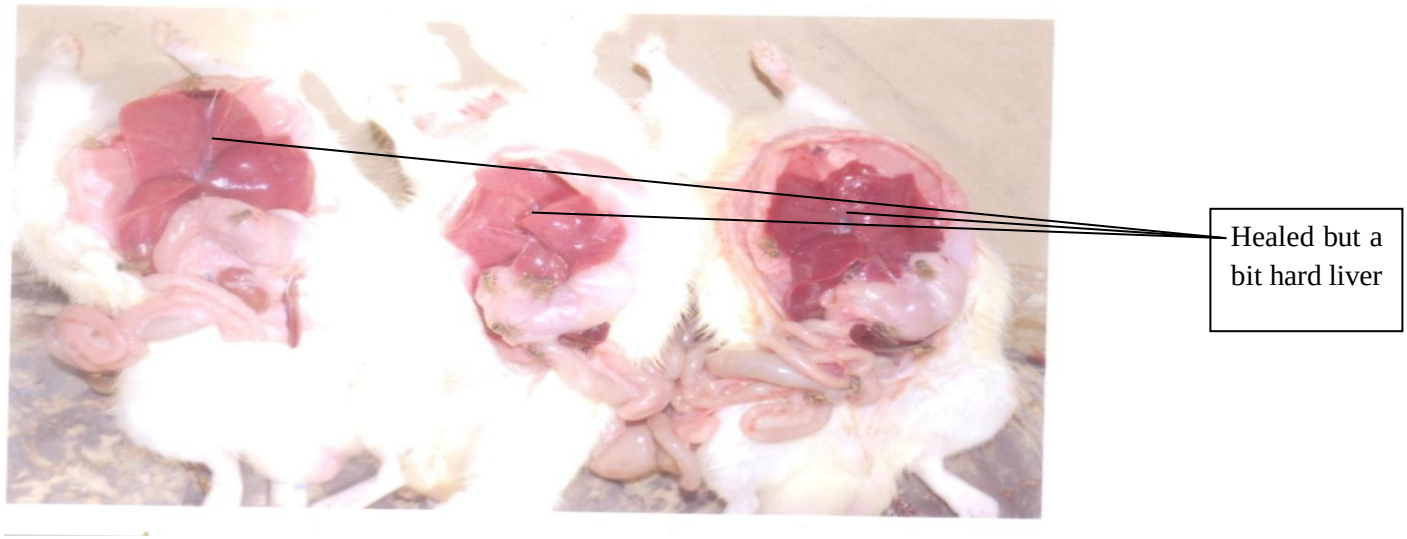

Figure 4: Liver of rats treated with 450mg/kg extract

Figure 5: Liver of rats treated with 210mg/kg extract

Table 4: Phytochemical analysis result

Secondary metabolite	Alkaloids	Flavonoids	Glycosides	Resins	Saponins	Steroids	Reducing sugar
Observation	+	+	+	-	+	+	-

Key: (+) = Present, (-) = Absent

Phytochemical screening is used to search for bioactive agents that could be used in the synthesis of very useful drugs [11]. The phytochemical analysis result (table 4) of the ethanolic root extract of *T. macroptera guill and perr* revealed the presence of alkaloids, flavonoids, saponins, glycosides resins, steroids etc. Saponins have drawn the attention of majority pharmaceutical chemist in the world. They are utilized in the synthesis of cortisones which is an anti-inflammatory drug. Also, they are widely used in the synthesis of sex hormones.

Carbon tetrachloride (CCl_4) is one common hepatotoxin used in the experimental study of liver diseases [12-14]. No animal died after 24 hours owing to the intraperitoneal administration of ethanol root extract at higher doses (Table 1). Though sign of toxicity noticed was general weakness, and loss of appetite. These signs increased as the dose raised to 5000 mg/kg. These signs were not visible in 10 mg/kg and 100 mg/kg doses respectively. LD_{50} greater than 5000 mg/kg b.w., is thought to be safe as suggested by Lorke (1983) [15]. This has been proved by the absence of any death recorded in any phase of the doses. Looking at figure 2, the liver of rats were actually injured and tissue when compared with those of figure 1, due to induction of carbon tetra chloride. Treatment of the injured liver (figures- 3, 4 and 5) with various doses of *T. macroptera* root extract revealed the liver returned physically to its normal shape and size. Comparing figure 1 and figure 2 it will be noticed that the liver of rats in figure 2 were tissue, ruptured, injured and bleeding as a result of injury caused by induction of carbon tetrachloride. Treatment of hepatic liver (figures 3, 4 and 5), with various doses of *T. macroptera* shows/ reveal that the extracts have anti hepatic tendencies. This is so, when figures 3, 4 and 5 are compared with figure 1. Figure 3 is liver of rats treated with the highest dose of ethanol extract of *T. macroptera* , they show no signs of bleeding and tissuing when compared with livers of rats induced with liver injury using carbon tetrachloride (figure 2) and looks almost if not exactly like the liver of those fed normally (figure 1). Liver of rats in figure 4 just like those of figure 3 returned to normal size and shape and showed no signs of bleeding but was a little bit hard in feeling when compared with livers in figure 3. Figure 5 showed no signs of bleeding when compared to figure 2 but did not return to the same size and shape when compared to figure 1. This may be due to fact that, the dose of the ethanol extract was least; 210 mg/kg

among the three extracts administered to treat the induced hepatitis. Though, *T. macroptera* is used to treat ailments in Nigeria, there is less or few scientific literature to support that.

Conclusion

The level at which the plant extract is termed toxic is yet to be confirmed. In conclusion, various parts of *T. macroptera* with different solvents should be explored on body weight after toxicity study. Also, post motem test and percentage organ body weight ratio. Currently, work is ongoing to isolate the active component(s).

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