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Hepatoprotective Effects of *Vitex Doniana* Fruit Extract on Carbon Tetrachloride Induced Liver Damage

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Abstract

The liver in the course of playing its role of detoxification, produces highly reactive molecules known as free radicals which can damage the liver. Hence, in spite of the advancement in the field of hepathology, liver disease is still on the increase. The need for a natural hepatoprotective agent motivated this study. Acute toxicity study and phytochemical screening of the ethanol fruit extract of *Vitex doniana* was done. The hepatoprotective study on the extract was carried out using adult albino rats. The liver damage was induced by the subcutaneous administration of carbon tetrachloride (CCl_4) in a single dose of 2 ml/kgbw per day for 4 days. The rats were then fasted for 36 h before the oral administration of the extracts (250, 500 and 1000 mg/kg) for 21 days. Effect of the extract on histopathology of the liver was also investigated.

A significant dose-dependent ($P<0.05$) reduction in the levels of ALT, AST and ALP enzymes was observed in the treatment groups when compared to the negative control. The extract significantly ($P<0.05$) increased the serum total protein when compared to negative control. Furthermore, a dose-dependent restoration of the liver at 100, 250, 500 and 1000 mg/kg dose of the fruit extract was observed. Considering the data obtained from this study, the fruit extract of Vitex doniana could possesse hypatoprotective property.

Keywords: Vitex doniana; Lliver damage; Hepatoprotective; Carbon tetrachloride

1. Introduction

The quest for efficacious remedies to treat various human ailments has centered on the utilization of medicinal plants [1]. As health care costs continue to escalate, the attraction for low-cost medicinal remedies has stimulated the interest of consumers to re-evaluate the potential of alternatives [2]. Some herbs have been employed in the treatment of liver ailments [3]. The liver is a vital organ that performs numerous functions essential for metabolism, immunity, digestion, detoxification, and vitamin storage [4]

Carbon tetrachloride (CCl_4) is a well-known hepatotoxin capable of causing liver damage [5]. Drug-induced liver injury accounts for approximately fifty percent of cases of acute liver failure globally [6], and approximately 29 million people worldwide suffer from chronic liver disease [7]. Some other causes of liver disease worldwide are chronic hepatitis B and C, alcohol and non-alcoholic steatohepatitis associated with obesity and metabolic syndrome [8].

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Disruption of the liver functions results in the excessive release of oxidants that damage hepatic cells. Additionally, activation of specific enzymes within the cytochrome P-450 system, particularly CYP2E1, contributes to oxidative stress [9]. Several liver function tests are available and, in most cases, the early signs of liver injury is elevation of hepatic enzymes.

Classes of drugs used in liver toxicity treatment include immunosuppressants[10], glucocorticoids[11], combination of therapeutic drugs[12], N-acetylcysteine [13] and glutathione [14].

Vitex doniana tree is a perennial tree often grown for its fruits. It is widespread in tropical Africa and is commonly known as Black plum or African olive, *Dinya* (Hausa), *Galbihi* (Fulani), *Oori-nla* (Yoruba), *Uchacoro/mbe mbe* (Igbo). It is widespread in the southwestern and southeastern Nigeria [15] with multiple uses such as food (both leaves and fruits) and medicines. It is commonly used in traditional settings for the management of a number of diseases; a decoction of the woody parts of the plant is used for the treatment of inflammatory disorders, stomach pains, diarrhea, rheumatic pains and dysentery [16]. The young leaves and roots are used for the treatment of wounds and male impotence respectively, and the fruit extract is used in the treatment of fatigue and constipation [17].

Figure 1 The fruits of *Vitex doniana*

2. Material and methods

2.1. Materials

2.1.1. Plant materials

Sample collection and Identification: The fresh fruits of *Vitex doniana* were purchased from Ekwulobia in Anambra state of Nigeria. The fruit was authenticated by a Taxonomist at the department of Botany, Nnamdi Azikiwe University, Awka, Nigeria, and a voucher specimen number (NAUBT 3218) was assigned to it.

2.1.2. Extraction of plant material

Extraction was carried out using a method that was previously described [18]. The fruits were cleaned to remove sand and other debris. After removing the thin mericarp, the fleshy juicy mesocarp was scraped off from the seeds and dried for a period of two weeks under room temperature. This dried mesocarp was grinded into powder with the help of an electrical grinder. About 800 g of the powdered material was cold macerated in 80 % ethanol. The mixture was agitated intermittently for three days (72 hours) before filtration. The filtrate was recovered and concentrated to dryness using water bath at 40 ^{0}C. The percentage yield of the extract was calculated and the extract stored in a refrigerator until when needed for experiments.

2.1.3. Animals

Thirty-five albino rats of both sexes weighing 130g-150g, purchased from the laboratory animal house of Chukwuemeka Odumegwu Ojukwu University, Igbariam, Nigeria, were used for the study. Ethical approval number PHACOOU/AREC/2023/032 was assigned to attest that the animals were cared for according to the Faculty of Pharmacy (COOU) Animal Research Ethics Committee Guidelines (PHACOOUAREC), which are in conformities with the National Institute of Health (NIH), USA, guidelines for the care and use of laboratory animals.

2.2. Methods

2.2.1. Qualitative phytochemical analysis:

Screening for the presence of secondary metabolites was carried out following the phytochemical tests as demonstrated earlier [19]. The presence of the following secondary metabolites were tested for: tannins, alkaloids, reducing sugars, flavonoids, glycosides, anthraquinones, saponins, acidic compounds and proteins.

2.2.2. Acute toxicity study:

The acute toxicity test was conducted using the up and down procedure (UDP) adopted [20] and revised [21]. Using this method, the animals were dosed one at a time and the doses were dependent on the response of the first animal to the initial dose. The second animal receives a lower dose if the first animal dies (the initial dose is decreased by a factor of 3.2) or the second animal receives a higher dose if the first animal survives (the initial dose is increased by a factor of 3.2). Three albino rats (130-150 g) were used as starting point. Two rats served as negative control having received 10 ml/kg of distilled water orally while the test animal received a default oral dose of 5000 mg/kg of the extract. The animals were then observed continuously for 4 hours for changes in behavior and for any other obvious signs of toxicity, and subsequently daily for additional 14 days for delayed toxicity.

2.2.3. Induction of liver damage

The liver damage was induced by the subcutaneous administration of carbon tetrachloride (CCl_4) in olive oil (50% v/v CCl4) in a single dose of 2 ml/kg body weight per day for 4 days. Thereafter, the rats were fasted for 36 h before the administration of the extracts.

2.2.4. Animal grouping and treatment

The rats were randomly divided into 7 groups (of 5 per group) as follows:

- Group 1: Normal control (received feed and water only)
- Group 2: Treated with olive oil and served as vehicle control
- Group 3: Treated with CCl_4 in olive oil (2 ml/kg body weight) (negative control)
- Group 4: Treated with CCl_4 in olive oil (2 ml/kg) + extract (250 mg/kg)
- Group 5: Treated with CCl_4 in olive oil (2ml/kg) + extract (500 mg/kg)
- Group 6: Treated with CCl_4 in olive oil (2ml/kg) + extract (1000 mg/kg).
- Group 7: Treated with CCl_4 in olive oil (2ml/kg) + vitamin E (100 mg/kg) (Standard drug)

2.3. Treatment of samples

At the end of 21 days of treatment with the fruit extracts, the animals were sacrificed by cervical capitation. Blood was obtained through cardiac puncture by means of hypodermal syringe and needle. The collected blood samples were placed in ice-cold micro centrifuge tubes. The blood was allowed to coagulate and centrifuged at 4000 r/min for 10 min. The serum samples were collected and utilized for biochemical parameters analysis.

2.3.1. Serum biochemical analysis:

Hepatic biochemical tests (AST, ALT, and ALP) were performed on a clinical chemistry automatic analyzer (ADVIA 2400, Bayer Diagnostics). AST, ALT, ALP, total protein, albumin, were measured according to the previous methods [22, 23] using commercially available assay kits (Bayer Diagnostics).

2.3.2. Histopathological study

Histological study of the liver specimen was carried out using the standard method [24]. The liver was instantly dissected from the animals after sacrificing and rinsed with normal saline. The liver tissues were treated individually

and fixed in 10% formalin for histological analysis. Using a microscope, a pathologist who was unaware of the study's procedure inspected the microscopic slides [25].

2.3.3. Statistical analysis

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS-25). Results were presented as mean ± standard error of mean (SEM) of sample replicates. Raw data were subjected to one-way analysis of variance (ANOVA) followed by post hoc turkey's test. $P<0.05$ was considered statistically significant.

3. Results

3.1. Yield of extract

The weight of the *V. doniana* fruit after the removal of the seeds was 765g and the crude extract obtained from it was 333g. The percentage yield was 43.8%.

3.2. Acute toxicity test (LD_{50}) of the extract:

Oral administration of the fruit extract up to 5000 mg/kgbw dose produced no changes in behavior, neither was there any mortality in any of the groups. Therefore, the LD_{50} of the fruit extract of *Vetex doniana* was above 5000 mg/kgbw.

3.3. Phytochemical screening of the extract:

Phytochemical analysis of the extract revealed abundant presence of alkaloids, tannins, flavonoids, glycosides, saponins, steroids, moderate amount of fats, acidic compounds and anthraquinone, and traces of reducing sugars (Table 1).

Table 1 Photochemical screening of extract

Constituents	Alk	Tan	Flav	Gly	Sap	Anthr	Ste	Fats	Acidic cpds	Red sugars
Presence	+++	+++	+++	+++	+++	++	+++	++	++	+

Key: Alk = alkaloids, Tan= tannins, Flav= flavonoids, Gly= glycosides, Anthr= anthraquinones, Sap=saponin, Ste= steroids, Red sugar=reducing sugar. (++) = moderately present, (+++) = abundant.

3.4. Effects of extracts on liver function parameters

At the end of 21-days of treatments with *V. doniana* fruit extract, there was a dose dependent significant ($P<0.05$) reduction in the levels of ALT, AST and ALP enzymes in the treated groups when compared to the negative control group (Table 2). The fruit extract was also able to significantly ($P<0.05$) increase the serum total protein level when compared to the negative control group (Table 2).

Table 2 Effects of the extract on liver function parameters

Group (n=5)	ALT (U/I)	AST(U/I)	ALP(U/I)	Total protein (g/L)	Albumin (g/L)
Normal control	14.50 ± 2.5*	46.11 ±1.45*	85.37±0.45*	69.14±0.40*	39.50±1.46*
Vehicle control	14.78±1.43*	45.55±2.30	82.88±1.37	66.15±0.27	39.99±0.21*
Negative control	39.42 ± 0.43	89.00±1.44	147.20±1.55	33.60±1.23	20.12±1.4
Positive control	14.36 ± 0.19*	46.29±0.12*	87.59±0.22*	68.27±0.88*	38.33±0.67*
250 mg/kg extract	20.12 ± 0.23*	55.55±1.29*	95.22±1.53*	59.11±1.59*	25.67±0.18*
500 mg/kg extract	16.21±1.11*	49.32±0.42*	88.44±0.47*	64.33±0.19*	49.59±1.33*
1000mg/kg extract	14.11±0.72*	45.79±0.33*	85.12±1.36*	68.67±1.14*	45.12±0.55*

Values are represented as Standard error of mean (SEM) (n=5). *p<0.05: Statistically significantly different from the control group

3.5. Histopathological study of the liver

The livers of the normal control and vehicle control groups (Figures A and B, respectively) revealed normal features of the liver. However, there was extensive degeneration of hepatocytes with focal necrosis, vacuolated cytoplasm (shown by the arrow), inflammatory cell infiltration and damaged central vein in the negative control group (Figure 2C). On the other hand, the fruit extract demonstrated a dose-dependent restoration of the liver at 100, 250, 500 and 1000 mg/kg doses (Figures 2D, 2E, 2F, and 2G respectively). At the end of the 21-day treatment, almost normal appearance of the hepatocytes around the central vein was observed in the 1000 mg /kg treated group (Figure 2G).

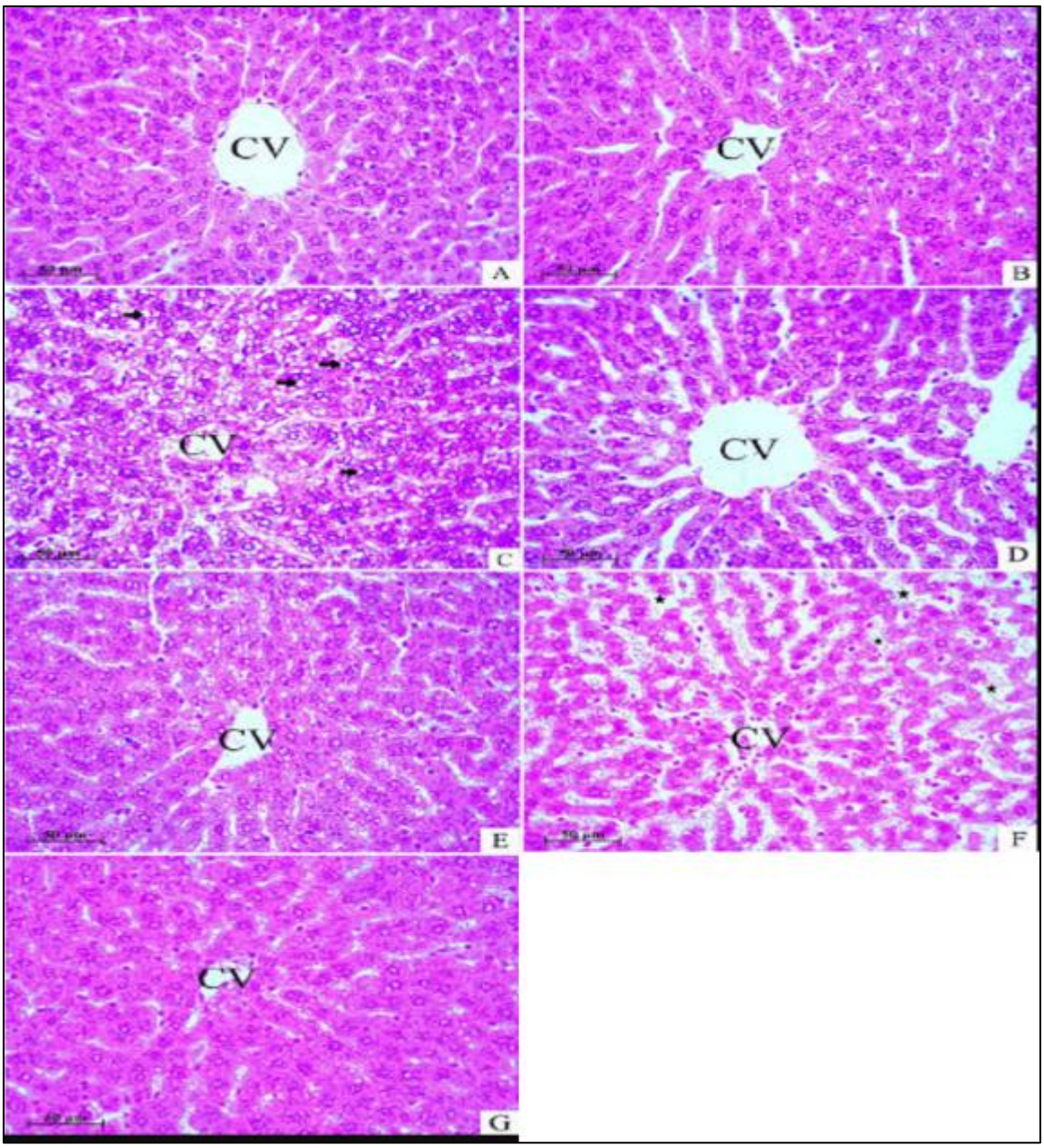

Figure 2 Histopathological study of the liver

4. Discussion

The liver is a vital organ that plays a crucial role in the maintenance of homeostasis. It is involved in detoxification, metabolism, storage and production [26]. Carbon tetrachloride (CCl_4) is a well-known hepatotoxin capable of causing liver damage [5]. The changes linked with CCl4-induced liver damage are similar to that of acute viral hepatitis, drug/chemicals-induced hepatopathy and oxidative stress. Carbon tetrachloride-induced hepatotoxicity model is commonly used for the evaluation of hepatoprotective efficacy of drugs and plant extracts [27]. Preserving the typical

physiological processes of the hepatic organs that have been disrupted by hepatotoxins is a solid criterion for evaluating the effectiveness of any hepatoprotective therapy.

The question of safety and toxicity places significant restrictions on the usage of therapeutic herbs. This is because some herbal medicines have been implicated in organ damage and fatal events [28]. In this study the acute toxicity study (LD_{50}) of *Vitex doniana* fruit extract revealed absence of toxic signs and deaths up to the dose of 5000 mg/kg in the rats. Absence of toxic signs and deaths in acute toxicity study are signs of relative safety.

The phytochemical screening of the fruit extract revealed the abundant presence of secondary metabolites such as alkaloids, tannins, flavonoids, glycosides, saponins, steroids and anthraquinone. Secondary metabolites have been linked to the pharmacological effects of plant extract (https://www.researchgate.net)

Vitex doniana fruit extract was able to significantly ($P < 0.05$) restore the elevated enzyme (AST, ALT and ALP) levels, while increasing the total serum protein in a dose dependent manner when compared to the negative control. Due to the destruction of hepatic cell wall these enzymes (AST, ALT and ALP) are drained into the blood stream. Increase in these enzymes suggests hepatic damage [29]. In contrast, total protein and albumin levels are lowered in the presence of hepatic damage [30]. The observed dose dependent significant ($P< 0.05$) decrease in serum enzymes in the treated groups may be due to the ability of the fruit extract to prevent the leakage of intracellular enzymes through membrane-stabilizing activity.

The result of the histopathology study of the livers revealed extensive damage in the liver of the negative control group, while there was a dose-dependent restoration of the liver of rats treated with 100, 250, 500 and 1000 mg/kg of the of the extract. At the end of the 21-day treatment, livers of group treated with 1000 mg/kg of extract displayed almost normal appearance of the hepatocytes. This histology result therefore further supports the hepatoprotective effect of the fruit extract of *Vitex doniana.*

5. Conclusion

The fruit extract of *Vitex doniana* possesses hepatoprotective effect against carbon tetrachloride induced liver damage, according to the findings of this study. However, further studies are required with the aim of demonstrating the precise molecular mechanisms of the hepatoprotective effects of *Vitex doniana* and determining its long-term toxicity profile.

Compliance with ethical standards

Disclosure of conflict of interest

The authors wish to confirm that there is no known conflict of interests associated with this paper and there has been no significant financial support for this work that could have influenced its outcome.

Statement of ethical approval

Ethical approval number PHACOOU/AREC/2023/032 was assigned to attest the animals were cared for according to the Faculty of Pharmacy (COOU) Animal Research Ethics Committee Guidelines (PHACOOUAREC), which are in line with the National Institute of Health (NIH), USA, guidelines for the care and use of laboratory animals.

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