Effects of Aqueous Stem Bark Extract of *Vitex doniana* (sweet) on Carbon Tetrachloride Induced Hepato-Toxicity in Albino Rats.

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Abstract: Traditional herbal medicine is an essential component in the treatment of various ailments and diseases in Nigeria. It is a common scenario due to its availability and affordability by majority of the population most of whom live in the rural areas. This study was conducted to verify the effectiveness of *Vitex doniana* in the treatment of hepatic disorders. The study indicated that the stem bark extract of *Vitex doniana* has hepatoprotective properties by decreasing the levels of the liver enzymes (ASAT and ALAT) in rats treated with 100mg/kg extract + CCl_4 and 150mg/kg extract + CCl_4 as compared with the groups treated with CCl_4 only. However, the decrease in enzyme activity (ASAT and ALAT) was not dose dependent based on this study. It was expected that the plant *Vitex doniana* will be further explored in order to discover means by which it can be used effectively in hepatic disorders.
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Key words: traditional herbal medicine, *vitex doniana* extract, hepatoprotective, ASAT and ALAT.

1. Introduction

Traditional herbal remedies are the first choice health care treatment for at least 80% of Africans and this stems round its various advantages of low cost, availability, accessibility, acceptability and perhaps its low toxicity (Elujoba et al., 2005). The uses of plant (root, stem, fruit and leaves) as well as animal and mineral materials for medicinal purposes have been reported, but there are no sufficient scientific data to confirm their efficacy (Sofowora, 1993). The history of drug discovery and even drug chemistry is exonarably bound to the plant kingdom and the process of deriving drugs from plant sources is certainly not new (Parfitt, 1978). There is therefore little or no doubt that herbal remedies have a critical role to play especially in this era of drug resistance. However, despite their medicinal values only little of these plants have been subjected to scientific verification and this has limited their introduction and use in orthodox pharmaceutical preparations.

Vitex doniana sweet is a plant native to, Nigeria, Botswana, Ethiopia, Kenya, Lesotho, Namibia, Niger, Senegal, Somalia, South Africa, Sudan, Tanzania, Uganda, and Zambia. It is locally called dinya (Hausa), Ucha koro (Igbo), oori-nla (Yoruba) (Burkill, 2000); Black plum, West African plum (En). Prunier noir, koro (Fr). Cetona (Po). Mfudu, mfuru, mfuu (Sw). (Ky, K.J.M., 2008); Galbihi (Fulani), Ngarmi (kanuri), Shika (Marghi).

Traditionally it is been used in the treatment of liver disease, it is also used to treat anaemia, jaundice, dysentery, leprosy and also supposed to improve fertility (Donmaydell, 1986). The root is used in treating gonorrhoea (FAO, 1983), the leaves are used as cattle feed and are rich in Vitamin A and B (Kapooria and Aime, 2005). The LD_{5o} intraperitoneally with 95% confidence limit of the water extract was estimated to be 980mg/kg (Abdulrahman et al., 2010).

Carbon tetrachloride is lipophilic and distributes in the lipid compartment of the body. The main routes of exposure of humans and animals to carbon tetrachloride include inhalation, ingestion, and absorption. On entry into the body, carbon tetrachloride causes a lot of injury to the organs of the body including the lungs, heart, gastrointestinal tract, kidneys, CNS, and liver (Reynolds et al., 1984), with the liver and kidney as major target organs of toxicity, in the liver, toxicity is manifested as steatosis (Fatty change of the liver parenchyma) followed by centrilobular necrosis (Boelsterli, et al. 1989). An initial step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes in the blood. Under normal circumstances, the enzymes reside within the liver cells, but when the liver is injured, these enzymes are spilled into the blood stream, the most sensitive and widely used are the aminotransferases (Kaneko, 1980).

This study therefore is to determine the hepato-protective effect of *vitex doniana* (sweet) stem bark extract against carbon tetrachloride induced liver damage in albino rats.

2. Materials and Method

2.1 Chemicals

All reagents used were obtained from RANDOX laboratories United Kingdom. These are buffer, RANDOX assay multisera level 2 and 3, sodium Hydroxide (NaOH), 2,4-dinitrophenylhydrazine, 10% formalin and Carbon tetrachloride (CCl_4) solution.

2.2 Sample Collection and Identification

Fresh stem bark twigs and flowers of *Vitex doniana* sweet were obtained from Damboa Local Government Area of Borno State, Nigeria. The plant was identified and authenticated by a taxonomist with the Department of Biological Sciences, University of Maiduguri.

2.2.1 Preparation of Extract

The stem bark OF *Vitex doniana* sweet were washed with distilled water, sun-dried and grounded into powder using pestle and mortar. One hundred grams (100g) of the powdered bark was placed in a flat bottom flask to which five hundred millilitres (500ml) of distilled water was added. This was heated at 100°C for 30 minutes, cooled and subjected to vigorous mixing and then filtered using whatmann filter paper size 0.1 μm . The filterate was concentrated to 0.1g/ml and stored at 4°C.

2.3 Experimental Animals

Thirty (30) white albino rats (*Rattus norvegicus*) of both sexes weighing between 100-280 grams were used. They were kept in plastic cages. The animals were allowed to adjust to the laboratory environment for one week before experimental procedures were commenced. The rats were feed with commercial chick mash (Vital feeds Jos, Nigeria) and allowed access to water ad-libitum.

2.3.1 Experimental Procedures

The rats were divided into six (6) groups of five (5) rats each, Group A, B, C, D, E, and F. Group A were given a single dose (4mg/kg) of carbon tetrachloride subcutaneously while group B were orally administered with the extract (100mg/kg) daily for seven days and carbon tetrachloride (4mg/kg) was given subcutaneously, 24 hours after the last dose of extract. In group C administration of the extract (150mg/kg) for seven days was carried out. 4mg/kg of carbon tetrachloride was given subcutaneously, 24 hours after the last dose of extract. Group D were administered 100mg/kg of the extract orally daily for seven days and group E were administered 150mg/kg of the extract orally daily for seven days. Finally group F which was the control group were given distilled water orally.

2.3.2 Collection of Blood

Five rats from each group were humanely sacrificed by severing the jugular vein and blood was collected in sets of plane test tubes; which was allowed to clot and was centrifuged at 1500rpm for five minutes. The serum collected was stored at 4°C. Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) values were used to assess the effect of various doses of the extract.

3. Results

3.1 Statistical Analysis

ALAT and ASAT determination was done using the method described by Reitman and Frankel, (1957) and Schmidt and Schmidt (1963). Results are presented as mean $\pm$ standard deviation and differences between means were assessed using analysis of variance (ANOVA).

Group B treated with CCl_4 after extract (100mg/kg body weight) administration, significantly $(P < 0.05)$ decreased the value of ALAT and ASAT when compared with those administered CCl_4 alone, while at 150mg/kg body weight extract administration before CCl_4 treatment, it is only the value of the ASAT that was significantly $(P < 0.05)$ decreased when compared with those treated with CCl_4 alone (Table 1).

Group A were administered CCl_4 (4mg/kg) alone and those administered the extract at 100 and 150mg/kg body weight before CCl_4 treatment recorded a significantly $(P < 0.05)$ higher values in ALAT than those administered distilled water, however the value of ASAT showed that only those treated with CCl_4 alone produced significant $(P < 0.05)$ increase when compared with those administered distilled water. Again, the groups treated with CCl_4 alone (group A) and those administered the extract at 100 and 150mg/kg body weight before CCl_4 treatment (group B and C respectively) produced significant $(P < 0.05)$ increase in both ALAT and ASAT when compared with those administered distilled water, group F and those administered the extract at 100mg/kg body weight, group D. Group B treated with CCl_4 after extract (100mg/kg body weight) administration produced significant $(P < 0.05)$ increase in ALAT when compared with E (those treated with 150mg/kg

body weight extract alone) while the value of ASAT showed that it is only those treated with CCl_4 alone (group A) that significantly ($P < 0.05$) increase when compared to those administered the extract 150mg/kg body weight alone (group E) table 1.

However, it is worthy of note that the extract alone produce a non significant ($P > 0.05$) decrease in the values of ALAT and ASAT over those administered distilled water, except at 150mg/kg body weight where the ASAT values was non significantly ($P > 0.05$) higher than those administered distilled water (table 1).

Table 1: The effect of aqueous extract of V*itex doniana* (sweet) stem bark on liver enzymes (mean± sd) of albino rats experimentally treated with CCl_4.

Group	Dose	liver enzymes	
		Alanine aminotransferase	aspartate aminotransferase
A	carbon tetrachlorideCCl_4 (4mg/kg)	129.0 ± 3.74	252.4± 28.11
B	100mg/kg Vitex doniana Extract and CCl_4	97.4±33.06*	183.8±51.75*
C	150mg/kg Vitex doniana Extract and CCl_4	115.2±8.35	187.2±11.45*
D	100mg/kg Vitex doniana Extract alone	32.6±9.39*a	115.0±20.11*a
E	150mg/kg Vitex doniana Extract alone	34.2±6.91*c	148.0±28.49*a
F	Distilled water alone	41.25±5.85*a	138.2±22.14*a

Key: - *- significant ($P < 0.05$) decrease compared to CCl_4 (4mg/kg)
a- significant ($P < 0.05$) decrease compared to groups administered 100 and 150mg/kg extract respectively before CCl_4 treatment.

4. Discussion

The result of the study showed that the stem bark extract of *Vitex doniana* administered at the dosage used and for the duration of the study decrease the level of liver enzymes that are normally liberated when the liver is diseased. There was a reduction in ASAT and ALAT levels in the rats treated with 100mg/kg extract + CCl_4 and 150mg/kg extract + CCl_4 as compared with the group treated with CCl_4 alone. The effect was however not dose dependent. Liver enzymes (ASAT and ALAT) are liberated into the blood whenever liver cells are damaged and Enzyme activity was reduced indicating that the extract did not have adverse effect on the liver. James *et al*., (2010) reported that aqueous extract of *Vitex doniana* may have anti-hepatotoxic effect against CCl_4-induced liver injury in rats. Ladeji and Okoye (1996) also reported that aqueous bark extract of *V. doniana* after CCl_4 administration significantly decreased serum levels of ASAT, ALAT, ALP and bilirubin, however, In contrast to our work was their report that the anti-hepatotoxic effect appears to depend on the dosage administered and on the duration of treatment, and administration of the same concentrations of aqueous extract of the plant prior to CCl_4 administration did not seem to offer any protection, but our work showed that prior administration of extract was able to protect the liver against CCl_4 induced hepatotoxicity. The presence of tannins and sapoinins in *Vitex doniana* extract (Sanni, 2002, Nwachukwu and Uzoeto; 2010) may be responsible for the hepato-protective effect of the plant. Studies have shown that saponins, especially terpene glycosides enhance natural resistance and recuperative power of the body (Sighn et al., 1991). Also, saponins are known to have inhibitory effects of various enzymes of the body (Sanni et al., 2005), which may further explain the hepatoprotective effect of *Vitex doniana*.

5. Conclusion

Vitex doniana has hepato-protective properties which justifies its use in the treatment of hepato-toxic disorders. However, there is need to carry out further researches on this plant in order to identify the specific saponins and tannins responsible for the suppression of liver-enzyme activity.

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