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Hepatoprotective Effect of Ethyl Acetate Extract of *Vitex doniana* Stem Bark on Carbon Tetrachloride (CCl_4) Induced Liver Damage in Wistar Rats

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ABSTRACT

The hepatoprotective effect of Ethyl Acetate Extract of *Vitex doniana* (EAEVD) stem bark on carbon tetrachloride (CCl4) induced hepatic damage was studied, to evaluate some biochemical parameters, to determine the *in vitro* antioxidant effect of the extract on Superoxide Dismutase (SOD), Catalase (CAT) glutathione peroxidase (GPX) and glutathione-s-transferase (GST) and to quantify the levels of some phytochemicals present in EAEVD stem bark. A total of twenty four Rats were used for the study. Animals in group1 served as vehicle control, Group 2 served as hepatotoxin (CCL_4) treated group, Group 3 served as positive control (Sylimarin) treated group, Group 4 was administered with 100 (mg kg^{-1} b.wt.) of the extract and group 5 was administered with 200 (mg kg^{-1} b.wt.) of the extract After the experimental period of 14 days. The animals were sacrificed, blood and liver samples were collected and used for the evaluation of the following biochemical parameters Aspartate amino transferase (AST), Alanine amino transaminase (ALT) and Alkaline phosphatase (ALP) in serum, as well as thiobarbituric acidreactive substances (TBARS), lipidhydroperoxides superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPX) and glutathione-s-transferase (GST) in the liver. Administration of 100 and 200 mg kg^{-1} b.wt. (EAEVD) significantly decreased ($p<0.05$) AST, ALT, ALP TBARS and lipid hydroperoxides with a significant increase ($p<0.05$) in the levels of SOD, CAT, GPX and GST in in group 4 and 5. Twenty five mg kg^{-1} b.wt. sylimar in was used as standard, The results show that the oral administration of EAEVD plant prevents the progression of hepatic damage in Ccl_4 treated wistar albino rats and suggest that the extract could be effective in the management of liver problems.

Key words: *Vitex doniana*, tetrachloromethanem, hepatotoxicity, Ethyl acetate, hepatoprotection

INTRODUCTION

Medicinal plants are greatly used to manage and treat diseased conditions especially in developing countries. Herbalists worldwide have used plants for the prevention and treatment of diseases. Because of their effectiveness and low cost, they were being prescribed even when the biologically active compounds they contain were not known (Bhawna and Kumar, 2010).

The liver plays an important functions amongst which are synthetic and secretory functions as well as metabolism of foreign compounds (Vuda *et al*., 2012). Viral hepatitis, alcoholism and exposure to toxic substances such as Carbon tetrachloromethane (CCl_4) are implicated in the liver damage which in turns influences the above physiological functions. Carbon tetrachloride is a potent hepatotoxin of environmental origin (Guven *et al*., 2003). It is a colourless liquid, non flammable and is denser than air that is commonly used for induction of experimental liver toxicity. This toxic chemical causes peroxidative degradation in the adipose tissue, resulting in infiltration of fats into the hepatocytes. It is metabolized by cytochrome P_{450} enzyme system to yield trichloromethyl radical (CCl_3) and trichloromethyl peroxy radical (CCl_3O_2) which are involved in the pathogenesis of liver (Recknagel, 1983; Okuno *et al*., 1986).

Reactive Oxygen Species (ROS) are continuously generated during metabolic processes to regulate a number of physico chemical functions essential to the body (Valko *et al*., 2007). They are potent electrophiles that are involved in nucleophilic substitution with a number of macromolecules such as nucleic acids, proteins etc.

Vitex doniana is a common medicinal plant in Nigeria. It is commonly known as black plum or African olive, Dinya in Hausa, Oori-nla inYoruba and Ucha coro in Igbo. However, the present study was designed to investigate hepatoprotective properties of ethyl acetate extracts of its stem bark in CCl_4 induced hepatic damage in Wistar albino rats.

MATERIALS AND METHODS

All chemicals and reagents used were purchased from sigma chemical company other reagents used were of analytical grade.

Collection, identification and extraction of plant material: The stem bark of *Vitex doniana* was obtained from the space of botanical garden, Ahmadu Bello University, Zaria, Nigeria in September, 2012 and authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University. Two hundred grams (200 g) of powdered plant materials were soaked in 1 L of Ethyl acetate at room temperature in a conical flask for 48 h. The suspension was filtered using cloth with fine pore and the filtrates were then concentrated in a crucible using a water bath set at 40°C.

Phytochemical analysis: Quantitative phytochemical analyses of Ethyl acetate extract of *Vitex doniana* (EAEVD) stem bark were carried out according to the methods below Tannins (Harborne, 1973), saponin and alkaloids (Obadoni and Ochuko, 2002) flavonoids (Boham and Kocipai, 1994).

Experimental animals: Adult Wistar albino rats weighing between 140-220 g of both sexes were obtained from the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. They were kept at the animal house in Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria and were allowed free access to commercial grower's mash (vital feed, Grend cereal PLC, Bukuru, Jos, Plateau Nigeria) and water *ad libitum*.

Acute toxicity test (LD 50): The acute toxicity test was carried out in accordance with the method of Lorke (1983). Nine rats were divided into three groups of three rats each in the first phase. They received 10, 100 and 1000 mg kg^{-1} b.wt. of the extract, respectively. In the second phase they were

divided into three groups of three rats each and received 1600, 2900 and 5000 mg kg b.wt., respectively. They were observed on daily basis throughout the study period for any toxicity associated sign.

Animal grouping and treatment: Twenty four Wistar rats of both sexes were divided into four groups of six rats each which include:

- (Negative control) fed with regular chow only
- Animals were treated with 1.2 mL kg^{-1} b.wt. CCI_4 only
- Animals were treated with CCI_4 and 25 mg kg^{-1} b.wt. sylimarin
- Animals were treated with 100 mg kg^{-1} b.wt. of (EAEVD) stem bark twice daily for 13 days and 1.2 mL kg^{-1} b.wt. of CCI_4 on the 14th day
- Animals were treated with 200 mg kg^{-1} b.wt. of (EAEVD) stem bark twice daily for 13 days and 1.2 m: kg^{-1} b.wt of CCI_4 on the 14th day

Collection of blood sample: After the experimental period of 14 days, the animals were anaesthetized in a diethyl ether saturated chamber and then dissected to expose the heart. Blood was obtained via cardiac puncture by means of a 5 mL hypodermic syringe and needle, the blood was placed in anticoagulant-free sample bottles and allowed to clot and then centrifuged at 3000 g for 10 min using (Uniscope model SM 902B Bench centrifuge, Surgi friend Medicals, England) each in order to obtain serum. Serum samples were collected for the estimation of AST, ALT and ALP and kept at -20°C until required.

Collection of liver sample: The liver of each rat was isolated and washed with chilled normal saline. Approximately 1 g was homogenized in 10 mL of 0.15 M KCl solution in an ice bath using a tissue homogenizer. The homogenate was centrifuged at 2000 g for 10 min at 4°C. The supernatant obtained was used for the estimation of SOD, CAT (TBARS), hydroperoxides, GPX and glutathione-s-transferase GST.

Determination of biochemical parameters: The activity levels of hepato-specific marker enzymes viz., AST, ALT in serum were estimated by the method of Reitman and Frankel (1957) and the activity level of ALP in serum was estimated by the method of King and Armstrong (1934) The level of lipid peroxidation in liver tissue was estimated by measuring the activity of malondialdehyde and other thiobarbituric acid reactive substances (TBARS) with thiobarbuturic acid (TBA) according to the method of Niehaus and Samuelsson (1968). The lipid hydroperoxides in the tissue were estimated by the method of Niehaus and Samuelsson (1968) The liver tissue levels of enzymatic antioxidants viz., SOD, CAT, GPX, Glutathione-s-transferase (GST) were estimated by the method of Kakkar *et al.* (1984), Sinha (1972), Rotruck *et al.* (1973) and Habig *et al.* (1974).

Statistical analysis: The results were expressed in Mean±Standard deviation. Statistical analysis was carried out by using one way (ANOVA). Duncan multiple comparison test in standard statistical software package of social science (SPSS).

RESULTS

The results of phytochemical analyses showed that alkaloid had the highest concentration (0.08±0.005), there was no significant difference ($p<0.05$) between alkaloid,

Table 1: Effect of ethyl acetate extract of *Vitex doniana* stem bark on the activities of AST, ALT and ALP in serum and TBARS, hydroperoxide, SOD, CAT, GPX and GST in liver

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Serum AST IU L^{-1} min^{-1} mg^{-1} protein	69.0±0.1500^{a}	108±0.50^{e}	76.1±0.11^{b}	92.7±0.28^{d}	87.8±0.25^{c}
ALT IU L^{-1} min^{-1} mg^{-1} protein	23.1±0.1500^{a}	67.2±0.15^{e}	32.3±0.26^{b}	43.4±0.25^{d}	39.2±0.25^{c}
ALP KA unit dL^{-1}	12.4±0.2500^{a}	33.9±0.15^{e}	16.5±0.15^{b}	25.3±0.32^{d}	19.3±0.10^{c}
Liver TBARS (nm 100 mg^{-1} tissue	0.53±0. 005^{a}	1.92±0.10^{d}	0.87±0.10^{b}	1.03±0.20^{c}	0.87±0.15^{b}
Hydroperoxide nm 100 mg^{-1} tissue	70.2±0.1000^{a}	120±0.26^{d}	79.4±0.20^{b}	96.4±0.20^{c}	96.1±0.15^{c}
SOD unit mg^{-1} protein	7.89±0.0100^{e}	2.38±0.01^{a}	5.81±0.05^{b}	5.23±0.15^{d}	4.46±0.15^{c}
CAT unit mg^{-1} protein	142.1±0.960^{e}	79.6±0.15^{a}	127±0.20^{d}	113±0.66^{b}	120±0.20^{c}
GPX unit mg^{-1} protein	12.7 ±0.100^{c}	6.60±0.01^{a}	10.2±0.10^{c}	9.77±0.02^{d}	9.87±0.02^{b}
GST unit 1 mg^{-1} protein	7.20±0.100^{e}	2.48±0.20^{a}	6.03±0.05^{d}	3.79±0.05^{b}	4.89 ±0.71^{c}

saponin (0.07±0.002) and flavonoids (0.07±0.005) content and also there was significant difference ($p<0.05$) between (0.06±0.05) and saponin content.

All values are expressed as Mean±S.D. of three replicates. Values with different alphabet superscripts along the row are significantly different at ($p<0.05$).

Aspartate amino transferase (AST), Alanine amino transaminase (ALT), Alkaline phosphatase (ALP) Thiobarbituric acid reactive substances (TBARS), Superoxide Dismutase (SOD), catalase (CAT) glutathione peroxidase (GPX), glutathione-s-transferase (GST).

Table 1 shows that the levels of serum AST ALT and ALP were significantly increased ($p<0.05$) to 108±0.50, 67.2±0.15 and 33.9±0.15 in CCl_4 only treated group (Group 2 when compared with group 1 (negative control). However, administration 100 and 200 mg kg^{-1} b.wt. of the extract showed significant reduction ($p<0.05$) in the level of AST ALT and ALP to 92.7±0.28, 43.4±0.25, 25.3±0.32 in group 4 and 87.8±0.25, 39.2±0.25, 19.3±0.10 in group 5 when compared with group 2. A significant increase ($p<0.05$) in the levels of hepatic tissue TBARS and lipid hydroperoxides 1.92±0.10 and 120±0.26 was recorded in group 2 when compared with group 1 as shown in Table 1. However, administration of the two doses of the extract significantly reduced ($p<0.05$) the levels to 1.03±0.20, 96.4±0.20 in group 4 and 0.87±0.15, 96.1±0.15 in group 5 when compared with group 1. The 200 mg kg^{-1} b.wt. of the extract group 5 had efficacy close to the standard drug (sylimarin) group 3 as there was no significant difference ($p<0.05$) in terms of reducing the levels of TBARS. Also there was no significant difference ($p<0.05$) between the two doses with respect to the reduction in the levels of hydroperoxides in group 4 and 5. The activity levels of liver antioxidant defense enzymes viz., SOD, CAT, GPX and GST significantly decreased ($p<0.05$) in the CCl_4 treated animals(group 2) to 2.38±0.01, 79.6±0.15, 6.60±0.01 and 2.48±0.20 when compared with group 1 as shown in Table 1. The oral administration of extract at the concentration of 100 and 200 mg kg^{-1} b.wt. significantly increased ($p<0.05$) the levels to 5.23±0.15, 113±0.66, 9.77±0.02 11.3.79±0.05 in group 4 and 4.46±0.15, 120±0.20, 9.87±0.02 and 4.89±0.71, in group 5 when compared with group 1. The Group 4 and 5 were also compared with group 3 (standard control) sylimarin treated groups.

DISCUSSION

The present study evaluates the hepatoprotective effect of (EAEVD) stem bark in CCl_4 induced hepatootxicity in rats. The activity levels of enzymes in the body fluids is being to serve as a useful indicator of a disease state. The enzymes AST and ALT catalyze the transamination reaction involving an amino acid and a keto acid to produce another amino acid and a keto acid

(Giboney, 2005). The ALP catalyses phosphorylation and dephosphorylation of DNA, RNA and proteins. It is most active at alkaline pH hence named so. The damage to the liver tissue and changes in cell permeability due to the effect CCL_4 might result in the leakage of AST, ALT and ALP into serum which is evidenced by their elevated levels (Madhumitha and Saral, 2009). Increased activity levels of AST, ALT and ALP in the serum of CCl_4 treated animals indicates a mixed hepatocellular damage . The significant decrease in the levels of the ALT, AST and ALP CCl_4 and EAEVD treated animals (Group 4 and 5) as shown in Table 1 might be due to decreased leakage from the liver cells . This suggests that the probable hepatic injury was repaired by the plant extract or restoration of the cellular permeability and thus reducing the severity of the toxic effect of CCl_4 in the liver tissue.

Excessive free radicals are being generated as a result of CCl_4 induced hepatotoxicity. Free radicals are the Reactive Oxygen Species (ROS) and they are known to cause oxidative damage to a number of molecules in the cell, including membranes-lipids, proteins and nucleic acids. The increased TBARS and hydroperoxide levels in the liver of CCl_4 treated animals (group 2) indicate tissue injury caused due to lipid peroxidation. During hepatotoxicity the oxidative stress marker enzymes catalase, SOD, GPX and GST are structurally and functionally impaired by free radicals resulting in liver damage. However, toxicity results from oxidative stress caused when the capacity of the cell to remove ROS is exceeded by the rate at which they are generated (Christen, 2000). The decreased activity of SOD in the liver tissue of CCl_4 treated animals (group 2) could be due to the oxidative inactivation of the enzymes or feedback inhibition due to excess Reactive oxygen species generation (Prabha and Annapoorani, 2009) and also the decreased CAT activity in the liver tissue of CCl4 treated animals group 3 might be due to enhance synthesis of O_2^- since oxygen radical is a powerful inhibitor of CAT (Popovic *et al.*, 2008). The perturbations in normal oxidative mechanism during CCl_4 administration could be thought to cause the decreased activity levels of GPX in the liver tissue. This agrees with the findings of Bharrhan *et al.* (2010) and Baravalia *et al.* (2011). The significant depletion of levels of TBARS and hydroperoxide in the liver tissue of the EAEVD treated animals (group 4 and 5) might be due to decreased lipid peroxidation or enahanced tissue antioxidant defense enzyme activity which indicates that the extract was able to reduce the free radical generation and restore free radical scavenging mechanism.

The significant increase ($p<0.05$) in the activity levels of SOD, CAT, GPX and GST in (group 4 and 5) is correlated with the increased free radicals scavenging mechanism. Similar reduction in lipid peroxidation could be due to increased antioxidant enzyme activity levels during plant extract supplementation (Rathee *et al.*, 2009).

The above study revealed that the Ethyl asetate extract of was able to protect CCl_4 induced hepatotoxicity in rats, significantly.

CONCLUSION

It can be concluded from the present study that, the ethyl acetate extract of *Vitex doniana* (EAEVD) stem bark could be efficiently used as hepatoprotective agents.

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