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Ameliorative effect of stem bark extract of *Piptadenia africana* on ethanol-induced toxicity in male wistar rats

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ABSTRACT

Alcohol consumption is a major culprit in the production of reactive oxygen species that enhances lipid peroxidation in the liver. In the present study, we evaluated the effect of daily oral administration of methanol stem bark extract of Piptadenia africana in ethanol induced hepatotoxicity. Thirty six male rats were randomly assigned into six groups. Ethanol toxicity was induced by exposing the rats to 45% ethanol (4.8 g/kg bw) administered by oral gavage. After treatment for 8 weeks, the animals were sacrificed and biochemical parameters like lipid profile, enzymatic and non-enzymatic antioxidants, indices of liver toxicity such as aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP), extent of lipid peroxidation as well as histopathological examination of the liver sections were evaluated. Chronic alcohol administration caused marked injury to liver cells as seen on the liver sections as well as elevated serum levels of AST, ALT and ALP with concomitant increase in triglycerides, total cholesterol, low-density-lipoprotein cholesterol (LDL-c) and malonydialdehde (MDA). Decrease in antioxidant activities as well as high-density-lipoprotein cholesterol (HDL-c) was also noticed. Rats pretreated with plant extract prior to ethanol exposure had decreased levels of AST, ALT, ALP, triglyceride, total cholesterol, LDLc, MDA and enhanced levels of enzymatic and non-enzymatic antioxidants as well as HDL-c. Intake of P. africana at the dosage of 100 or 200 mg/kg bw in this study was able to ameliorate the toxic response to chronic ethanol consumption in rats and this could be attributed to its bioactive constituents.

Keywords: Ameliorate, Ethanol, liver, lipid peroxidation, *Piptadenia africana*.

INTRODUCTION

The liver has been implicated to plays crucial metabolic roles such as protein production, synthesis of enzymes, regulation of cholesterol and blood clotting as well as blood detoxification and purification [1]. Alcohol is metabolized primarily in the liver and some of the byproducts generated during alcohol metabolism may be more toxic than alcohol itself and may contribute to the development of alcohol liver disease (ALD) [2]. Alcohol is the major culprit of liver disease in western countries and this arises from excessive ingestion of alcohol [3]. Alcoholic liver disease (ALD) is triggered by complex interactions between metabolic intermediates of alcohol, inflammation and immune responses from cellular injury [4, 5]. Hepatocytes are the primary site of alcohol detoxification, its major toxic metabolic intermediate, acetaldehyde could cause damage to hepatocytes as well as form adducts with proteins and DNA [6, 7]. Thus, alcohol affects many organs of the body, but perhaps most notably affected are the

central nervous system and the liver. Almost all ingested alcohol is metabolized in the liver and excessive alcohol consumption may lead to acute and chronic liver disease [8, 9].

ALD has attracted the attention of researchers all over the world, the underlying mechanisms are not fully understood but there is evidence of the involvement of oxidative stress in its development [10]. Previous studies have demonstrated that ethanol-induced liver injury is associated with enhanced lipid peroxidation, protein carbonyl formation, formation of the 1-hydroxyl ethyl radical, formation of lipid radicals and decreases in hepatic antioxidant defense capabilities, especially glutathione [11]. Treatment with antioxidants such as vitamin E, vitamin C, and other agents that enhance hepatic antioxidant capacity have been tried in an attempt to deal with the ethanol-induced liver injury [12 - 15].

Piptadenia africana symonmys *Piptadeniastum africanum* belongs to the family Leguminoseae and the genius has several other species such as *P. peregrina, P. macrocarpa, P. excelsus, P. rigida, P. vidiflora, P. parguayersis* [16]. The species are distributed throughout Tropical Africa, however only *P. africana* is found in Western Cameroun. *P. africana* is also known as African oak (English), Dahoma (Ghana), Atui (Cameroun), Dabema (Ivory Coast), Agboin and Ekhimi (Nigeria). They are found in mixed deciduous and evergreen forest, it often stands as a single tree on farmlands [16]. The leaves and fruits are used by traditional healers as aphrodisiac, tonic, dysmenorrhea, enema, urethritis and as an abortificient. Decoctions of the root bark are used to treat cough, bronchitis, pneumonia, urinary tract infections, leprosy, headaches and mental disorders. The pottice from the root bark is used externally for haemorrhoids, tooth ache and rheumatism. The stem bark extract is used for gastric pain and fever. The leaves are also used to expel worms internally and it is applied externally to treat oedema, skin complaints and rheumatism. Non curative of the root and stem barks include as arrow poison, fish poison and as rodenticide. The stem bark are used for gastric pain, fever by the local population of Noun division, Cameroon. Compounds isolated from the stem bark include betulinic acid, sitosterol, 24(S)-stigmat-5,22-dien-3β-O-glucopyranoside, 5,6-dimethoxy-7-hydroxyflavone, antiquol B, β-amyrine and new lactone derivatives piptadenol A,B and C [17 - 18]. This lead us to evaluate the effect of continuous administration of the stem bark extract on acute ethanol-exposed rats for 8 weeks and to evaluate its effect on serum markers of liver toxicity, lipid profile, antioxidant status and histopathological examination of liver sections.

MATERIALS AND METHODS

2.1. Chemical

Diagnostic Kits for cholesterol, triglycerides and high-density lipoprotein – cholesterol (HDL-C) precipitants were procured from Boehringer Mannheim Diagnostical (Mannheim, Germany).1-Chloro-2,4-dinitro benzene (CDNB), 5,5'-dithiobis-(2-dinitrobenzene) (DTNB), Adrenaline, Thiobarbituric acid (TBA), Reduced glutathione (GSH) and bovine serum albumin (BSA) were purchased from Sigma Chemical (St Louis, MO, USA). Randox diagnostic kit was used for ALT, AST and ALP. Other reagents used were of purest quality available commercially available.

2.2. Plant Collection and extraction.

Stem bark extracted was collected from Western Cameroun by our colleague Mr. Ramsey Kandem, who also sent it for authentication at the herbarium. It was air dried at room temperature in Chemistry Department of University of Doula, Cameroon and ground to fine powder using Hammer mill and extracted by maceration in cold using methanol for 72hrs. Extract was filtered and the filterate was concentrated on a Bucchi rotary evaporator R114 to give a dark brown solid. The methanoic extract was carefully scraped from the flask into an evaporating dish and was left to dry in vacuum in fume cupboard. From 1 kg of air dried ground stem bark gave a yield of 88.7 g (11.27%) of the dark brown methanolic extract. 1g of crude extract was sent for phytochemical screening and the remaining.

2.3. Animals

Thirty six male, albino rats (Wistar strain), weighing between 105 - 125g were procured from Primate colony of the Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria and were housed in the Animal house, Biochemistry Department, University of Ibadan, Ibadan at normal room temperature. The rats were acclimatized for two weeks to standard laboratory conditions, temperature 25-35 °C, under a 12hr light/dark cycle in standard rat cages, on standard diet (pelletized Guinea feed, purchased from Guinea Feed, Ibadan, Nigeria). The animals were allowed free access to food and water *ad libitum*. Rats were randomly distributed into six groups of six animals each. Group A_1 (PA 100 mg/kg bw alone), Group A_2 (PA 100 mg/kg bw and 45% ethanol), Group B_1 (PA

200 mg/kg bw alone), B_2 (PA 200 mg/kg bw and 45% ethanol), Group C (positive control on standard pellet diet and saline solution only) and Group D (Negative control on 45% ethanol and saline solution only). Saline solution was used as vehicle for the administration of extracts and rats were administered daily dose orally for 8 weeks during which the animals were observed daily for feeding habit, psychomotor changes and mortality. Animal experiments followed protocols established by the National Institute of Health (NIH) (NIH publication 85-23, 1985) for the Care and Use of Laboratory Animals and all procedures involving rats were conducted according to the ethical guidelines approved by the Animal Ethical Committee of Afe Babalola University (ABUAD-SCIREC03/13/09/057).

2.4. Sample Collection

Twenty four hours after the last administration of the extract or ethanol; the animals were sacrificed by cervical dislocation. Blood was obtained using 2 ml syringe by cardiac puncture into clean bottles without anticoagulant and were left to stand for 1 h for complete coagulation. The clotted samples were spun at 3000 rpm for 10 minutes, the supernatant serum was removed and it was stored at 4 ^{0}C. The visceral organ (liver) were quickly removed, washed with 1.15% KCl, homogenized in 56 mM Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride and the homogenate was centrifuged at 10,000 rpm for 15 minutes at 4^{0}C. Supernatant was stored until needed. Small pieces of liver sections were fixed in 10% formal saline and sent to Veterinary Anatomy Department, University of Ibadan, Ibadan for histopathological examination.

2.5. Biochemical assays

Quantification of the protein was by Biuret method Gornal *et al*., [19] with bovine serum albumin (BSA) as standard. Lipid peroxidation was assayed by measuring thiobarbituric acid reactive substances (TBARS), by colorimetric reaction of the lipid peroxidation product malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a pink precipitate, which was read at 532 nm by spectrophotometry as described by Varshney and Kale [20]. Catalase (CAT) activity was done by measuring the rate of decomposition of hydrogen peroxide at 570 nm as described by Sinha [21]. Reduced glutathione (GSH) level was determined by measuring the rate of formation of chromphoric product in a reaction between DTNB (5,5´-dithiobis- (2-nitrbenzoic acid) and free sulphydryl groups at 412 nm [22]. GPx was determined by the method of Hafeman *et al*., [23] based on the degradation of H_2O_2 in the presence of GSH. Microsomal glutathione-s-transferase (GST) activity was determined using CDNB at 340 nm, superoxide dismutase (SOD) activity was assayed using the method of Misra and Fridovich [24], Cholesterol was determined using Randox kit. The lipoproteins were assayed using enzymatic colorimetric method for very low density lipoprotein (VLDL) and low density lipoprotein (LDL) by precipitation using phosphotungistic acid and magnesium chloride. After centrifugation at 3000 g for 10 min at 25 °C, the clear supernatant contained high density lipoprotein (HDL) fraction using HDL-cholesterol precipitant kit. The LDL-cholesterol (LDL-c) was calculated using the formulae of Friedwald *et al*., [25]. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase were assayed using Randox kits.

2.6. Statistical analysis

All values were expressed as the mean ± S.D of six animals. Data were analyzed using one-way analysis of variance (Anova) followed by the post-hoc Duncan multiple test for analysis of biochemical data using SPSS (10.0) statistical software. P Values < 0.05 were considered statistically significant.

RESULTS

3.1. Phytochemical screening

Phytochemical analysis revealed the presence of carbohydrates, flavonoids, terpenoids and sponins in *Piptadenia africana* (PA) stem bark as shown on Table 1

3.2. Effect of PA and Ethanol on Lipid Profile in Rats.

Table 2 shows the data obtained for the effect of PA and ethanol on lipid profile in rats. Ethanol consumption caused a significant increase in triglyceride compared to control; these were significantly ameliorated by administration of plant extract. The groups on plant extract only had triglyceride levels lower than control animals on regular rat chow. There was slight difference in LDL-c but it was none statistically different in this study and Group D (negative control) had a slightly lower value than Group C rats (positive control) on rat chow only. Total cholesterol was significantly increased by ethanol intake and the elevated serum cholesterol levels were significantly ameliorated by the plant extract, Groups A_1 and B_1 on plant extract alone had a lower cholesterol levels than the

positive control rats (Group C). HDL-c was significantly increased in groups on both plant extract and ethanol compared to ethanol only group.

3.3. Effect of PA on lipid peroxidation, enzymatic and non-enzymatic antioxidants

Tables 3 presents the data obtained for lipid peroxidation, enzymatic and non-enzymatic antioxidants in this study. Ethanol consumption caused a decrease in antioxidant levels (SOD, CAT, GSH, and GST) compared to control with a concomitant increase in LPO level. Co-administration of PA with ethanol lowered the elevated level of LPO and reverted the hepatic antioxidants status.

3.4. Effect of PA on serum ALP, ALT and AST activities

Ethanol administration caused significant increase in serum ALP, AST and ALT (Table 4). Animals treated with PA had their elevated serum ALP, AST and ALT activities reverted when compared to group D (ethanol only).

3.5. Histopathological examination of liver sections

Histopathological report is shown on Figs 1. Chronic alcohol consumption showed signs of fatty liver with accumulation of fat droplets.

Table 1: Result of the qualitative phytochemical screening of stem bark of PA

Phytochemical screened for	Present or not
Alkaloids	Absent
Tanin	Present
Saponin	Present
Flavonoids	Present
Anthraquinone	Absent
Phenol	Present
Carbohydrates	Present

Table 2: Effect of PA and Ethanol on Lipid Profile in Rats (mean ± S.D., n = 6).

Groups	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)
Group A_1	214.09±3.71	246.65±4.2	173.82±9.62	34.28±2.21
Group A_2	294.54±3.93	269.46±2.45^{ab}	178.27±8.35^{a}	27.17±6.93^{ab}
Group B_1	194.04±8.84	239.76±1.98^{ab}	167.67±7.76	35.73±4.78^{ab}
Group B_2	287.42±5.28^{b}	264.80±3.57^{b}	172.59±3.98	32.01±6.09
Group C	232.58±5.77	250.84±3.28	177.13±5.32	37.28±3.40
Group D	346.07±6.00^{a}	284.09±6.83^{a}	181.87±6.21^{a}	26.01±9.82^{a}

Values are mean of six animals ±SD, [a]The mean is significantly different compared to control at (P<0.05) and [b] mean is significantly different compared to ethanol only group

Table 3: Effect of PA on lipid peroxidation, enzymatic and non-enzymatic antioxidants (mean ± S.D., n = 6)

Groups	LPO (unit/mg protein)	GSH (µg/ml)	GST (µm/min/mg protein)	SOD (units/mg protein)	CAT (units/mg protein)
Group A_1	14.72±2.76^{c}	27.46±4.25	7.65±3.99	38.10±6.23^{b}	36.53±6.88
Group A_2	16.99±4.87^{ab}	25.79±8.36^{b}	5.49±2.87	17.88±4.06^{ad}	34.37±4.74
Group B_1	14.11±3.31	30.54±8.14	8.31±3.11	47.62±3.88	41.35±4.39^{b}
Group B_2	16.66±3.33	26.00±6.14	6.39±2.72^{a}	32.14±8.11	34.51±3.50^{d}
Group C	13.86±7.03	26.79±.99	6.74±8.32^{b}	35.71±9.77	34.91±3.98
Group D	19.05±5.54^{a}	20.83±3.62^{a}	3.03±0.39^{ad}	16.67±3.11^{a}	27.33±4.80^{a}

Values are mean of six animals ±S.D; [a] Significantly different compared to control (P<0.005) [b]Significantly different compared to ethanol (P<0.005); [c]Significantly different compared to group A2 (P<0.005) [d]Significantly different compared to group B1 (P<0.005)

Table 4: Effect of PA on serum ALP, ALT and AST activities (mean ± S.D., n = 6)

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Group A_1	90.50±2.10	36.40±9.66	25.30±3.50
Group A_2	102.00±8.24^{b}	38.90±5.22^{b}	39.68±2.11^{ab}
Group B_1	86.17±4.49	32.00±3.33	15.64±9.78
Group B_2	94.17±2.46^{bc}	38.47±2.99^{bc}	38.64±5.05
Group C	91.42±3.11	38.00±8.99	33.12±7.08
Group D	129.08±1.24^{a}	75.30±6.21	56.12±4.88^{a}

Values are mean of six animals ±S.D; [a] Significantly different compared to control (P<0.005)
[b]Significantly different compared to ethanol (P<0.005); [c]Significantly different compared to group A2 (P<0.005) [d]Significantly different compared to group B1 (P<0.005)

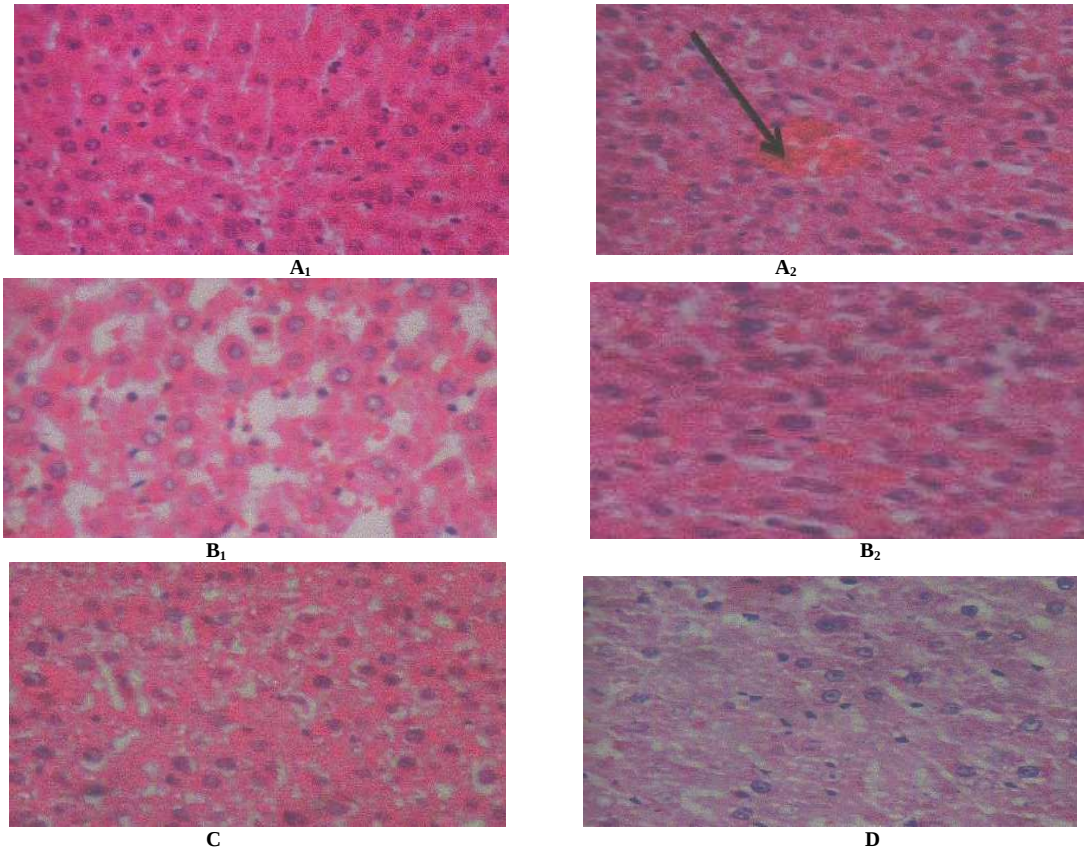

Fig. 1. Histopathological examination of liver sections. Liver tissues were stained with H&E (×400)

A_1: There is moderate congestion of the central veins
A_2: There is widespread foamy appearance of hepatocytes with marked congestion of sinusoids and central vain.
B_1: There is a moderate multifocal dissociation of the hepatic cords and individualization of hepatocyte.
B_2: There is moderate random single cell necrosis of hepatocytes.
C: There is no visible lesion seen
D: There is widespread vacuolar change of the hepatocytes.

DISCUSSION

Hepatocytes are exposed to reactive oxygen species (ROS) during alcohol metabolism in the liver. Acute and chronic exposure to high level of ROS may result in an imbalance state which could disrupt the redox state of the hepatocytes detoxifying enzymes leading to increased lipid peroxidation. Flavonoids have been reported to inhibit lipid peroxidation in the cell membrane and modulate lipid fluidity [26]. Phytochemicals present in methanol stem bark extract of PA with possible antioxidant activities are the flavonoids [18].

The groups on plant extract only had triglyceride (TG) levels lower than control animals on regular rat chow. This is in agreement with earlier report [27, 28]. Generally, levels of TG, total cholesterol (TC) and LDL-c were reduced by PA in all treated groups when compared with the ethanol only group. HDL-c was significantly increased in groups treated with PA extract and ethanol when compared with ethanol only group. Thus treatment of rats with PA extracts at a dose of 100 or 200 mg/kg bw prior to ethanol administration offered protection in modulating serum lipids. [29, 30].

Lipids are a heterogenous group containing active metabolic substances which play an important role in the pathogenesis of alcoholic liver disease. Ethanol is a powerful indicator of hyperlipidemia in both animals and humans. The most common lipid abnormalities during chronic alcohol consumption are known to produce hypercholesterolaemia and hypertriglyceridaemia [31 - 34]. Brown *et al*., [35] reported that increase in serum TC and LDL-c concentrations, and liver dysfunction after exposure to xenobiotics may be due to the effect of LDL

receptors or oxidized cholesterol toxicity caused by free radicals generated, beside the major metabolic consequence of oxidative stress, that associated with the deposition of triglycerides in liver [36].

Animals treated with PA and ethanol had reduced values of LPO and increased values of the antioxidants, thus PA was able to modulate oxidative stress caused by ethanol intake. Similar report has been made [37 - 39]. It is now well accepted that progression of liver injury is a multfactorial event that involves a number of genetic and environmental factors. Among these factors is growing interest in the role of free radical-mediated oxidative stress. Lipid peroxidation (LPO) mediated by free radicals is considered to play a pivotal role in the mechanism by which ethanol may exert its toxic effects on the liver and other extra hepatic tissues. Increased LPO and decreased antioxidant status are reflection of the liver's susceptibility to ethanol-induced oxidative damage [30, 40].

SOD catalysis the reaction superoxide anion radical to hydrogen peroxide and catalase reduces it to water, GST is involved in biotransformation of xenobiotics while GSH plays an important role in the antioxidant effects, nutrient metabolism and regulation of cellular events [41]. The lowered activities of these antioxidants would lead to the accumulation of reactive oxygen species or increased lipid peroxidation which could have deleterious effect on membrane integrity and function [42].

Animals treated with PA had their elevated serum ALP, AST and ALT activities reverted compare to group D animals (ethanol only). The lowered values indicate that PA confers protection against tissue damage. Serum biomarker enzymes like ALP, AST and ALT are stored in the liver cells and increase in the levels of these enzymes in serum indicates damage to the liver cells [43, 44]. Histopathology of liver sections showed animals intoxicated with ethanol showed prominent sinusoids, periportal degeneration of hepatocytes with cellular infiltration of mononuclear cells unlike control rats with normal liver morphology and no visible lesions Fig 1. Continual administration of PA for 8 weeks produced moderate congestion of the central veins, while animals co-administered PA and ethanol showed widespread foamy appearance of hepatocytes and mild to moderate multifocal dissociation of hepatic cords and individualization of hepatocytes. Histopathological changes observed were in agreement with results obtained from various biochemical estimations.

In conclusion, on the basis of the results obtained in the present study, methanol stem bark extract of PA at a dose of 100 or 200 mg/kg bw exhibits significant hepatoprotective activities against oxidative damage induced by chronic consumption of ethanol.

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