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Research Article

Pretreatment of albino rats with aqueous leaf extract of *Ziziphus mauritiana* protects against alcohol-induced liver damage

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Abstract

Purpose: *The effect of the aqueous extract of Ziziphus mauritiana leaf on hepatic lipid peroxidation, reduced glutathione and total antioxidant status was studied in chronic alcohol-induced liver damage.*
Method: *Alcohol-induced liver toxicity was created by oral administration of 40% alcohol solution (v/v, 1ml/100g) to rats for 6 weeks. Other groups of rats were pretreated with 200 and 400 mg/kg bw aqueous extracts of Ziziphus mauritiana leaf or 100 mg/kg bw silymarin (reference drug) 30 min prior to alcohol ingestion. Body weight of rats was monitored weekly. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and liver total antioxidant status, reduced glutathione and lipid peroxidation were evaluated.*
Result: *Rats that received alcohol only showed significantly ($p<0.05$) elevated levels of ALT, AST, bilirubin, and hepatic lipid peroxidation while reduced glutathione, total antioxidant status and body weight significantly ($p<0.05$) decreased compared to control rats. Pretreatment of rats with aqueous extract of Ziziphus mauritiana 30 min prior to alcohol administration resulted in significant ($p<0.05$) depression of ALT, AST, bilirubin and lipid peroxidation levels compared to the group exposed to alcohol only. Administration of Ziziphus mauritiana extract prior to alcohol ingestion significantly ($p<0.05$) resulted in increased levels of reduced glutathione and total antioxidant status compared to the group that received alcohol only.*
Conclusion: *The results of this study indicate that the aqueous extract of Ziziphus mauritiana leaf may prevent chronic alcohol-induced liver injury by enhancing the levels of total antioxidant status and inhibiting hepatic lipid peroxidation.*

Key words: Lipid peroxidation, reduced glutathione, alcohol, *Ziziphus mauritiana*

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INTRODUCTION

Chronic alcohol consumption increases the capacity of cytochrome P450 2E1 (CYP2E1) to oxidize ethanol up to 10-fold which consequently increases the prooxidative burden[1]. Reactive oxygen species (ROS) generated during ethanol oxidation via CYP2E1 contributes to ethanol-induced liver injury[2]. Although the pathogenesis of alcohol-induced liver disease remains the subject of debate[3], one factor that has been suggested as playing a central role in many pathways of alcohol-induced damage, and which has been the focus of much research is the excessive generation of these free radicals, which can result in a state called oxidative stress[4]. Numerous studies have indicated that excessive ethanol intake induces the mass production of free radicals in the body, which are considered to be associated with alcoholic liver disease[5]. The most important characteristic of toxic free radicals either *in vivo* or *in vitro* is peroxidation of lipids resulting in tissue damage and death of affected cells[6]. Several reports have implicated free radical-induced lipid peroxidation in the pathogenesis of alcohol-induced liver toxicity[7,8]. Antioxidants play an important role in the protection of cells and tissues against free radical-mediated tissue injury[9]. Although significant progress has been made in understanding the pathogenesis of alcoholic liver diseases, current therapies for these diseases are not effective. At present, except for abstinence from alcohol intake, there is no effective modality of either prevention or treatment[10, 11]. Antioxidants of plant origin have been reported to either inhibit or prevent the development of fundamental cellular disturbances resulting from excessive alcohol consumption [10].

Ziziphus mauritiana belongs to the family *Rhamnaceae*. The ripe fruit of the plant is mostly consumed raw, but is sometimes stewed. The young leaves are eaten in Indonesia; also the leaves are applied as poultices and are claimed to be helpful in liver troubles, asthma and fever[12, 13]. Recently the hepatoprotective activity of ethanol extract of *Ziziphus mauritiana* leaf against carbon tetrachloride (CCl_4) -induced liver damage in rats and the antidiarrhoea activity of the methanol root extract were reported[14, 15]. This study was designed to evaluate the chronic alcohol-induced liver oxidative damage and the efficacy of pre-treatment of aqueous extract of *Ziziphus mauritiana* leaf on chronic alcohol-induced liver damage.

MATERIALS AND METHODS

Materials

Fresh leaves of *Ziziphus mauritiana* were collected 20km along Yola-Mubi highway, Adamawa State, Nigeria in the month of July 2005. The leaves were shed-dried under room temperature at 30 ± 2°C. The dried material was ground into powder using mortar and pestle and sieved with a sieve of 0.3mm aperture size (Endicott Ltd, London). A voucher specimen of the plant has been deposited (BCDD-03b) in the Department of Biochemistry Federal University of Technology, Yola. 100g of the powdered plant material was steeped in 600 ml of distilled water and heated in water bath for 3 hours at 90°C. The mixture was allowed to cool to room temperature and filtered. The filtrate was later freeze-dried yielding a residue corresponding to 22.56 ± 1.72g/100g. 200 or 400 mg/kg body weight (bw) of the extract was used to pre-treat rats 30 min before alcohol administration.

Experimental Design

Thirty six male Wistar albino rats weighing between 100-120 g were purchased from the animal house of Faculty of Medical Sciences, University of Jos. The animals were kept under standard condition of 12/12 h dark-light circle and were fed with standard feed (Grand Cereals and Oil Mills Ltd, Jos) and water *ad libitum*. The animals were allowed to acclimatize for two weeks prior to distribution into different groups of six rats each and treated for six weeks as follows:

Group I – rats in addition to normal diet were given normal saline (1ml/100g bw).

Group II – rats in addition to normal diet received 40% alcohol solution (v/v, 1ml/100g bw, p.o).

Group III, IV and V rats in addition to normal diet were pretreated with either 200, 400mg/kg bw aqueous extract of *Ziziphus mauritiana* leaf or 100 mg/kg bw silymarin (reference

hepatoprotective material) respectively 30 min before feeding rats with 40% alcohol solution (v/v, 1ml/100g bw).
Group VI – rats were administered 400 mg/kg bw aqueous extract of *Ziziphus mauritiana* in addition to normal diet. In all the groups body weight was monitored weekly.

Methods

Biochemical estimations

At the end of the treatment period, rats were sacrificed under light ether anesthesia and blood collected via the ocular vein without the use of anticoagulant. The blood was allowed to stand for 10 min before being centrifuged at 2,000 rpm for 10 min to obtain serum for analysis. The levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TB) were assayed using Randox clinical test kits (Randox Ransod Laboratories, Ltd U.K.). The rats were dissected, the liver removed and placed in iced beakers. A 1g portion of the liver was used to prepare homogenate of the liver (10%) in ice cold KCl solution (1.15% w/v) using Teflon homogenizer. The homogenate was centrifuged at 4000g for 10 min to remove debris. The supernatant was used for the estimation of reduced glutathione – GSH[16], and total antioxidant status - TAS (Randox, Ransod Ltd, UK).
Lipid peroxidation was determined by the thiobarbituric acid (TBA) reaction method[17]. Briefly, liver samples were removed from sacrificed rats and placed in ice-cold 0.15M KCl solution in a beaker embedded in salinated ice. The liver samples were rinsed thoroughly in the saline solution and excess fluid blotted out with a paper towel before weighing the samples in chilled containers. One gram portion of the liver was homogenized in 4ml ice-cold 0.15M KCl solution using a homogenizer. Liver homogenate (2ml) was treated with 1ml 2% TCA and then 2ml 0.6% TBA in a ground glass tube. The lightly-stopper tube was warmed for 10min in a boiling water bath. The mixture was centrifuged at 3000 x g for 10minutes to remove precipitated proteins. The absorbance of a pink colour produced by the reaction was read at 530nm against water blank.

Statistical Analyses

Results were presented as Mean ± Standard Deviation. Statistical analyses was carried out using student't' test to compare for significant difference between two means. Significant difference was considered at $p<0.05$ with the aid of Microsoft Excel 2003.

RESULTS

Table 1 shows the result of body weight changes of rats pre-treated with different concentrations of aqueous extract of *Ziziphus mauritiana* leaf or silymarin for 6 weeks. There was a significant ($p<0.05$) decrease in the body weight of rats that ingested alcohol only compared to normal group at the end of week 4. By week five however, rats pre-treated with 400mg/kg bw aqueous extract of *Ziziphus mauritiana* or 100mg/kg bw silymarin significantly gained body weight compared to group ingested with alcohol only. This indicates that pre-treatment with *Ziziphus mauritiana* extract or silymarin decreased weight loss due to chronic alcohol ingestion.
Table 2 presents the result of pre-treatment on enzyme and non-enzyme markers of tissue damage. Exposure of rats to alcohol for six weeks significantly ($p<0.05$) elevated the levels of AST, ALT, ALP and TB. Pre-treatment of rats with 200 or 400mg/kg bw aqueous extract of *Ziziphus mauritiana* leaf significantly ($p<0.05$) and dose dependently decreased the levels of AST, ALT, ALP and TB.
The results for the estimation of reduced glutathione, total antioxidant status and lipid peroxidation are presented in Table 3. Both the levels of reduced glutathione and total anti-oxidant status were significantly ($p<0.05$) lowered in group ingested with alcohol only. However, groups pretreated with 200 or 400mg/kg bw of the aqueous extract of *Ziziphus mauritiana* or silymarin had significantly ($p<0.05$) elevated levels of reduced glutathione and total antioxidant status. Lipid peroxidation as assayed by thiobarbituric acid reactive substances (TBARS) was significantly ($p<0.05$) elevated in group ingested with alcohol only compared to normal group. This effect was however, significantly lowered by pre-treatment with the extract of *Ziziphus mauritiana* or silymarin.

Table 1: Effect of pre-treatment with *Ziziphus mauritiana* aqueous leaf extract on body weight changes in chronic alcohol fed rats (g/week)

Week	Normal	Alcohol	200mg/kgZm + Alc	400mg/kgZm + Alc	100mg/kgSily + Alc
1	18.2 ± 2.4	17.81 ± 1.9	17.48 ± 1.7	17.69 ± 1.5	18.3 ± 2.4
2	26.48 ± 2.1	24.36 ± 2.4	23.57 ± 1.9	24.68 ± 2.1	24.32 ± 1.8
3	35.61 ± 3.2	32.43 ± 2.8	34.26 ± 3.1	35.24 ± 2.8	33.47 ± 2.7
4	48.57 ± 3.4	38.27 ± 3.1[a]	42.26 ± 2.4[a]	44.13 ± 3.1	45.12 ± 4.2
5	59.03 ± 4.2	43.32 ± 3.7[b]	48.34 ± 2.8 [b]	52.56 ± 3.4	56.38 ± 3.7
6	68.41 ± 3.8	50.18 ± 4.1[c]	58.67 ± 4.3[c]	63.5 ± 3.9	68.54 ± 4.2

Results are Mean ± S.D (n = 5). [a]Significantly lower than control group at week 4 (p<0.05). [b]Significantly lower compared to normal at week 5 (p<0.05). [c]Significantly lower compared to normal rats at week 6 (p<0.05).

Table 2: Effect of Pre-treatment with *Ziziphus mauritiana* aqueous leaf extract on serum markers of tissue damage in chronic alcohol fed rats

Treatment	AST (U/L)	ALT (U/L)	TB (mg/dl)
Normal	26.04 ± 2.88	20.76 ± 1.77	0.011 ± 0.002
Alcohol	69.14 ± 4.85[d]	38.30 ± 2.55[d]	0.635 ± 0.03[d]
Zm 200mg/kg + Alc	48.13 ± 2.74[e]	33.02 ± 1.09[e]	0.045 ± 0.007[e]
Zm 400mg/kg + Alc	34.16 ± 2.31[ef]	27.00 ± 2.03[ef]	0.140 ± 0.001[ef]
Sily 100mg/kg + Alc	32.58 ± 2.90[ef]	24.94 ± 1.17[ef]	0.097 ± 0.004[ef]

Results are Mean ± S.D, (n = 5). [d]Significantly higher compared to control group (p<0.05). [e]Significantly lower compared to experimental group (p<0.05). [f]Significantly lower compared to group pretreated with 200 mg/kg bw extract.

Table 3: Effect of *Ziziphus mauritiana* aqueous leaf extract on liver total antioxidant status, reduced glutathione and lipid peroxidation

Treatment	TAS (mMol/g)	GSH (µMol/g)	LP (nMol/mg)
Normal	15.25 ± 1.07	18.40 ± 1.90	2.12 ± 0.19
Alcohol	6.11 ± 1.24 [α]	8.05 ± 1.12 [α]	9.06 ± 1.15[d]
Zm 200mg/kg + Alc	9.33 ± 1.07[β]	11.24 ± 1.83 [β]	6.02 ± 1.25[e]
Zm 400mg/kg + Alc	12.05 ±1.17 [βc]	14.85 ± 1.58 [βc]	4.08 ± 0.43[ef]
Sily 100mg/kg + Alc	13.93 ± 1.14 [βc]	15.52 ± 1.34 [βc]	3.07 ± 0.32[ef]

Results are Mean ± S.D, (n = 5). [α]Significantly lower than control (p<0.05). [β]Significantly higher compared to experimental group (p<0.05). [c]Significantly higher compared to group pretreated with 200 mg/kg bw extract (p<0.05). [d]Significantly higher compared to control group (p<0.05). [e]Significantly lower compared to experimental group (p<0.05). [f]Significantly lower compared to group pretreated with 200 mg/kg bw extract.

DISCUSSION

Chronic consumption of alcohol, which is rich in energy (7.1 cal/g), does not produce any gain in body weight[18]. Substantial use of alcohol has profound effect on nutritional status which may cause primary malnutrition by displacing other nutrients in the diet of high energy content or because of associated medical disorders. Secondary malnutrition results due to maldigestion or malabsorption of nutrients caused by gastrointestinal complications[19]. Pre-treatment of rats with aqueous extract of *Ziziphus mauritiana* leaf prior to alcohol feeding significantly increased their body weight gain compared to rats administered alcohol only. Pre-treatment with 400 mg/kg bw aqueous extract of *Ziziphus mauritiana* leaf prevented decrease in body weight gain similar to the group pretreated with silymarin. Other researchers earlier reported significantly decreased body weight gain due to alcohol compared to control rats[20, 21].

The levels of total antioxidant status and reduced glutathione were found to significantly decrease in rats that ingested alcohol only compared to normal rats. Decreased antioxidant status shown during ethanol intoxication has been attributed to oxidative stress formation[22]. Increased reactive oxygen species formation produces cytotoxic oxidative stress and increased lipid peroxidation[23]. These processes phenomenon ultimately result in depletion of hepatic antioxidants leading to the development of hepatic diseases in response to ethanol ingestion.

Cellular glutathione is a major component of the intracellular reducing machinery and a crucial factor of apoptosis[24]. Reduced glutathione acts as an antioxidant both intracellularly and extracellularly in conjunction with various enzymatic processes that reduce hydrogen peroxide and hydroperoxides by oxidizing reduced glutathione to its oxidized form and other mixed disulfides[25]. Ethanol administration has been reported to induce loss of glutathione from the liver and to cause a decrease in its hepatic content[26]. Glutathione, a tripeptide containing a sulfhydryl group, is a highly distinctive amino acid derivative with an important role in defending against lipid peroxidation. Since reduced glutathione inhibits lipid peroxidation in the liver, the decrease in GSH content in the liver could well give rise to an increase in malondialdehyde (MDA) formation, thus explaining the enhanced peroxidation in liver of ethanol-treated rats. Other factors that may contribute to a decrease in tissue-reduced glutathione include glutathione synthesis, utilization and limited intracellular reduction of oxidized glutathione to its reduced form[7]. Pre-treatment of rats with aqueous extract of *Ziziphus mauritiana* leaf prior to alcohol administration significantly prevented the depletion of the total antioxidant status and glutathione levels of rats compared to rats that ingested alcohol only. Increased levels of total antioxidant status and glutathione could be the result of direct stimulation of antioxidant enzyme synthesis and increased levels of other antioxidants in the liver or enhanced reduction of oxidized glutathione. The availability of sufficient amount of reduced glutathione may enhance the detoxification of active metabolites through the involvement of glutathione peroxidase[27].

Chronic alcohol ingestion has been reported to result in increased lipid peroxidation through the formation of 4-hydroxy 2,3 neonenal, 4-hydroxy 2-3-alkanals and malondialdehyde[22]. Increased lipid peroxidation could be a direct consequence of decreased hepatic reduced glutathione content, which gives rise to an increase in malondialdehyde formation[8]. Escalation of lipid peroxidation in the liver due to chronic alcohol ingestion may be the result of increased formation of free radicals as well as the inhibition of antioxidant enzymes such as superoxide dismutase and catalase[28].

Decreased lipid peroxidation due to pretreatment with aqueous extract of *Ziziphus mauritiana* leaf may be a reflection of the *in vivo* elevated levels of antioxidants. Increased level of reduced glutathione is known to protect against lipid peroxidation[9].

CONCLUSION

Pretreatment of rats with aqueous extract of Ziziphus mauritiana was found to protect rats from chronic alcohol-induced liver injury and weight loss. The action of the extract was made possible through inhibition of lipid peroxidation

and subsequently increasing the total antioxidant status of the liver.

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