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Aqueous Ziziphus mauritiana leaf extract pretreatment shields albino rats' livers against alcohol-induced damage.

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Abstract

Goal: In chronic alcohol-induced liver damage, the impact of an aqueous extract of Ziziphus mauritiana leaf on hepatic lipid peroxidation, decreased glutathione, and overall antioxidant status was investigated. Method: Rats were given an oral 40% alcohol solution (v/v, 1 ml/100 g) for six weeks in order to cause liver damage. Before consuming alcohol, rats in other groups received pretreatment in the form of 200 and 400 mg/kg bw aqueous extracts of Ziziphus mauritiana leaf or 100 mg/kg bw silymarin (reference medication) 30 minutes beforehand. Rats' body weight was recorded once a week. The following biomarkers were assessed: reduced glutathione, lipid peroxidation, total bilirubin, aspartate aminotransferase (AST), and liver total antioxidant status.Results: Compared to control rats, animals given alcohol alone had considerably ($p<0.05$) higher levels of ALT, AST, bilirubin, and hepatic lipid peroxidation, and significantly ($p<0.05$) lower levels of glutathione, total antioxidant status, and body weight. Rats who received an aqueous extract of Ziziphus mauritiana thirty minutes before being administered alcohol showed a substantial ($p<0.05$) decrease in their levels of ALT, AST, bilirubin, and lipid peroxidation when compared to the group that simply received alcohol. When compared to the group that only received alcohol, the administration of Ziziphus mauritiana extract before alcohol consumption substantially ($p<0.05$) raised levels of reduced glutathione and overall antioxidant status.
In conclusion, the study's findings suggest that by raising total antioxidant status levels and preventing hepatic lipid peroxidation, an aqueous extract of Ziziphus mauritiana leaf may protect against long-term alcohol-induced liver damage.

INTRODUCTION

Chronic alcohol use increases cytochrome P450 2E1's (CYP2E1) ability to oxidize ethanol by up to ten times, which raises the prooxidative burden1. Ethanol-induced liver damage is partially caused by reactive oxygen species (ROS) produced by CYP2E1 during ethanoloxidation2. The excessive production of these free radicals, which can lead to a state known as oxidative stress4, has been proposed as a factor that plays a central role in many pathways of alcohol-induced damage and has been the focus of much research, even though the pathogenesis of alcohol-induced liver disease is still up for debate3. Several studies have shown that consuming too much ethanol causes the body to produce large amounts of free radicals, which are thought to be linked to alcoholic liver disease5. The primary feature of harmful free radicals, both in vivo and in vitro, is the peroxidation of lipids, which causes tissue damage and cell death in the afflicted cells. Numerous studies have linked the etiology of alcohol-induced liver damage to lipid peroxidation caused by free radicals.7,8. Antioxidants are crucial for defending tissues and cells against tissue damage caused by free radicals. Even though our knowledge of the pathophysiology

of alcoholic liver disorders has advanced significantly, our present treatments for these conditions are ineffective. There is now no effective method of prevention or therapy available, with the exception of abstaining from alcohol consumption10, 11. It has been shown that antioxidants derived from plants may either suppress or stop the development of basic cellular abnormalities brought on by excessive alcohol use 10.

Ziziphus mauritiana is a member of the Rhamnaceae family. The plant's mature fruit is typically eaten uncooked, however it may sometimes be boiled. In Indonesia, the young leaves are consumed. They are also used as poultices and are said to be beneficial for fever, asthma, and liver problems12, 13. Recently, it was revealed that the methanol root extract had antidiarrhea properties and the ethanol extract of Ziziphus mauritiana leaf had hepatoprotective properties against liver damage in rats caused by carbon tetrachloride (CCl4).

MATERIALS AND METHODS

Materials

In July 2005, fresh Ziziphus mauritiana leaves were gathered 20 kilometers along the Yola-Mubi route in Adamawa State, Nigeria. The leaves were shed and allowed to dry at 30 ± 2oC room temperature. Using a mortar and pestle, the dry material was crushed into a powder, and then it was sieved using a 0.3 mm aperture size sieve (Endicott Ltd, London). The Federal University of Technology, Yola's Department of Biochemistry contains a voucher specimen of the plant (BCDD-03b). After steeping 100g of the powdered plant material in 600 ml of distilled water, it was heated in a water bath at 90°C for three hours. After letting the mixture settle to normal temperature, it was filtered. After the filtrate was freeze-dried, the residue was 22.56 ± 1.72g/100g. Rats were pre-treated with the extract at 200 or 400 mg/kg body weight (bw) 30 minutes prior to the administration of alcohol.

Design Experiments

36 male Wistar albino rats, weighing between 100 and 120 g, were bought from the University of Jos's Faculty of Medical Sciences' animal house. The animals received standard feed (Grand Cereals and Oil Mills Ltd, Jos) and unlimited water while being housed in a typical 12/12 h dark-light cycle. The animals were given two weeks to acclimate before being divided into six-rat groups and receiving the following six-week treatment:

Group I: Rats received normal saline (1 ml/100 g bw) in addition to their regular diet.

Group II: Rats were given a 40% alcohol solution (v/v, 1ml/100g bw, p.o.) in addition to their regular food.

In addition to receiving their regular meal, Group III, IV, and V rats received a pretreatment of 200, 400 mg/kg bw aqueous extract of Ziziphus mauritiana leaf or 100 mg/kg bw silymarin (reference hepatoprotective substance) 30 minutes prior to the rats being fed a 40% alcohol solution (v/v, 1ml/100g bw).

In addition to their regular food, Group VI rats received an aqueous extract of Ziziphus mauritiana at a dose of 400 mg/kg bw. Every week, the body weight of each group was recorded.

Techniques

Biochemical approximations

Rats were slaughtered at the conclusion of the treatment period while under a mild ether anesthetic, and blood was drawn from the ocular vein without the use of an anticoagulant. In order to extract serum for examination, the blood was centrifuged at 2,000 rpm for 10 minutes after being let to stand for 10 minutes. Using Randox clinical test kits (Randox Ransod Laboratories, Ltd., U.K.), the levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total

bilirubin (TB) were measured. After the rats were dissected, the liver was taken out and put in ice-filled beakers. Using a Teflon homogenizer, a piece of the liver weighing 1g was homogenized (10%) in an ice-cold KCl solution (1.15% w/v). To get rid of debris, the homogenate was centrifuged for 10 minutes at 4000g. The total antioxidant status (TAS) and reduced glutathione (GSH16) were calculated using the supernatant (Randox, Ransod Ltd, UK).

The technique of reaction with thiobarbituric acid (TBA) was used to evaluate lipid peroxidation17.

In short, liver samples from rats that had been slaughtered were taken out and put in a beaker that was submerged in salted ice, along with an ice-cold 0.15M KCl solution.

Before the liver samples were weighed in cold containers, they were properly washed in the saline solution and any excess fluid wiped off with a paper towel. Using a homogenizer, one gram of liver was homogenized in four milliliters of ice-cold 0.15M KCl solution. In a powdered glass tube, liver homogenate (2 ml) was treated with 1 ml 2% TCA and 2 ml 0.6% TBA. The mildly-stopper tube was heated in a bath of boiling water for ten minutes. For ten minutes, the mixture was centrifuged at 3000 x g to eliminate any precipitated proteins. At 530 nm, the absorbance of the pink color the reaction generated was measured against a blank of water.

Analyses Statistical The findings were shown as Mean ± Standard Deviation. The student "t" test was used in statistical analysis to check for a significant difference between two means. Using Microsoft Excel 2003, a significant difference was defined as $p<0.05$.

RESULTS

The body weight changes of rats that were pre-treated for six weeks with varying quantities of aqueous extract of Ziziphus mauritiana leaf or silymarin are shown in Table 1. By the conclusion of the fourth week, the body weight of the rats that drank alcohol alone had significantly ($p<0.05$) decreased in comparison to the normal group. In contrast to the group that consumed alcohol alone, the rats that received a pretreatment of 400 mg/kg bw aqueous extract of Ziziphus mauritiana or 100 mg/kg bw silymarin acquired a substantial amount of body weight by week five. This suggests that pre-treatment with silymarin or Ziziphus mauritiana extract slowed the weight loss caused by long-term alcohol use. The outcome of the pre-treatment on enzyme and non-enzyme indicators of tissue injury is shown in Table 2. Rats' levels of AST, ALT, ALP, and TB were considerably ($p<0.05$) raised after six weeks of alcohol exposure. Rats' pre-treatment with 200 or 400 mg/kg bw aqueous extract of Ziziphus mauritiana leaf resulted in a dose-dependent and statistically significant ($p<0.05$) reduction in their AST, ALT, ALP, and TB levels.

Table 3 displays the findings for the estimate of reduced glutathione, total antioxidant status, and lipid peroxidation. In the group that consumed alcohol alone, there was a substantial ($p<0.05$) decrease in both the levels of reduced glutathione and total antioxidant status. On the other hand, groups that were pretreated with 200 or 400 mg/kg bw of the aqueous extract of silymarin or Ziziphus mauritiana exhibited substantially ($p<0.05$) higher levels of total antioxidant status and reduced glutathione.

When comparing the group that consumed alcohol alone to the normal group, there was a substantial ($p<0.05$) increase in lipid peroxidation as measured by thiobarbituric acid reactive substances (TBARS). However, pretreatment with Ziziphus mauritiana or silymarin extract considerably reduced this impact.

Table 1: Pretreatment with aqueous leaf extract of Ziziphus mauritiana and changes in body weight (g/week) in chronic alcohol-fed rats

Week	Normal	Alcohol	200mg/kgZm + Alc	400mg/kgZm + Alc	100mg/kgSily + Alc
1	16.2 ± 2.4	17.81 ± 1.9	17.48 ± 1.7	17.69 ± 1.5	16.3 ± 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 ± 2.68	20.76 ± 1.77	0.011 ± 0.002
Alcohol	69.14 ± 4.85[a]	38.30 ± 2.55[a]	0.635 ± 0.03[a]
Zm 200mg/kg + Alc	48.13 ± 2.74[a][c]	33.02 ± 1.09[c]	0.045 ± 0.007[a][c]
Zm 400mg/kg + Alc	34.16 ± 2.31[a][c]	27.00 ± 2.03[a][c]	0.140 ± 0.001[a][c]
Sily 100mg/kg + Alc	32.58 ± 2.90[a][c]	24.94 ± 1.17[a][c]	0.097 ± 0.004[a][c]

Results are Mean ± S.D. (n = 5). [a]Significantly higher compared to control group ($p<0.05$). [c]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[a]	8.05 ± 1.12[a]	9.06 ± 1.15[a]
Zm 200mg/kg + Alc	9.33 ± 1.07[a][c]	11.24 ± 1.83[a][c]	6.02 ± 1.25[a][c]
Zm 400mg/kg + Alc	12.05 ± 1.17[b][c]	14.85 ± 1.58[b][c]	4.08 ± 0.43[b][c]
Sily 100mg/kg + Alc	13.93 ± 1.14[b][c]	15.52 ± 1.34[b][c]	3.07 ± 0.32[b][c]

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

DISCUSSION

Alcohol has a high energy content (7.1 cal/g), however chronic use does not increase body weight18. Heavy alcohol use has a significant negative impact on nutritional status and may lead to primary

malnutrition by displacing other nutrients in a diet rich in energy or by causing related medical conditions. Maldigestion or inadequate nutritional absorption brought on by gastrointestinal issues leads to secondary malnutrition19. Rats given an aqueous extract of Ziziphus mauritiana leaf before being fed alcohol showed a considerable increase in body weight gain in comparison to rats given alcohol alone. Similar to the group pretreated with silymarin, pretreatment with 400 mg/kg bw aqueous extract of Ziziphus mauritiana leaf prevented reduction in body weight increase. Alcohol-induced body weight increase was much lower in these rats than in control rats, according to previous research20, 21.

Rats that consumed alcohol alone showed a considerable reduction in reduced glutathione and overall antioxidant status when compared to normal rats. Oxidative stress production has been linked to a decrease in antioxidant status during ethanol intoxication22. Higher lipid peroxidation and cytotoxic oxidative stress are caused by increased reactive oxygen species formation23. When ethanol is consumed, these mechanisms eventually cause the liver's antioxidant reserves to be depleted, which causes liver disorders to develop. One of the main elements of the intracellular reducing mechanism and a critical component in apoptosis is cellular glutathione24. By oxidizing reduced glutathione to its oxidized form and other mixed disulfides, reduced glutathione functions as an antioxidant both intracellularly and extracellularly in combination with other enzyme activities that decrease hydrogen peroxide and hydroperoxides25. It has been shown that the injection of ethanol causes the liver to lose glutathione and reduces its hepatic content26. A very unique derivative of amino acids, glutathione is a tripeptide with a sulfhydryl group that plays a crucial function in protecting against lipid peroxidation. Rats given ethanol treatment had increased liver peroxidation, which might be explained by an increase in malondialdehyde (MDA) generation due to decreased glutathione, which suppresses lipid peroxidation in the liver. A decrease in tissue-reduced glutathione may also be caused by diminished intracellular reduction of oxidized glutathione to its reduced form7, glutathione production, and utilization. animals who were pre-treated with an aqueous extract of Ziziphus mauritiana leaf before being given alcohol showed a substantial reduction in the depletion of their glutathione levels and overall antioxidant status when compared to animals that were given alcohol alone. Elevated levels of glutathione and overall antioxidant status may stem from accelerated elimination of oxidized glutathione, higher amounts of other antioxidants in the liver, or direct stimulation of antioxidant enzyme production. Glutathione peroxidase27 may be more effectively involved in the detoxification of active metabolites when there is an adequate supply of reduced glutathione available.

It has been shown that long-term alcohol use increases lipid peroxidation by forming malondialdehyde22, 4-hydroxy 2-3-alkanals, and 4-hydroxy 2,3 neonenal. Decreased hepatic reduced glutathione level may directly lead to increased lipid peroxidation, which in turn causes an increase in malondialdehyde formation8. Chronic alcohol use may cause the production of more free radicals and the inhibition of antioxidant enzymes like catalase28 and superoxide dismutase, which may lead to an increase in lipid peroxidation in the liver.

Reduced lipid peroxidation after pretreatment with Ziziphus mauritiana leaf aqueous extract might be a result of the high antioxidant levels seen in vivo. It is well recognized that elevated reduced glutathione levels protect against lipid peroxidation9.

CONCLUSION

Rats that were pretreated with an aqueous extract of Ziziphus mauritiana were shown to be shielded against weight loss and chronic alcohol-induced liver damage. The extract's impact was enabled by its suppression of lipid peroxidation, which in turn raised the liver's overall antioxidant state.

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