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Hepatoprotective effect of aqueous extract of *Aframomum melegueta* on ethanol-induced toxicity in rats

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In recent years there have been remarkable developments in the prevention of diseases, especially with regards to the role of free radicals and antioxidants. Ethanol-induced oxidative stress appears to be one mechanism by which ethanol causes liver injury. The protective effect of aqueous plant extract of *Aframomum melegueta* on ethanol-induced toxicity was investigated in male Wistar rats. The rats were treated with 45% ethanol (4.8 g/kg b.w.t.) for 16 days to induce alcoholic diseases in the liver. The activities of alanine aminotransferase, aspartate aminotransferase and triglyceride were monitored and the histological changes in liver examined in order to evaluate the protective effects of the plant extract. Hepatic malondialdehyde and reduced glutathione, as well as superoxide dismutase and glutathione-*S*-transferase activities were determined for the antioxidant status. Chronic ethanol administration resulted in a statistically significant elevation of serum alanine aminotransferases and triglyceride levels, as well as a decrease in reduced glutathione and superoxide dismutase which was dramatically attenuated by the co-administration of the plant extract. Histological changes were related to these indices. Co-administration of the plant extract suppressed the elevation of lipid peroxidation, restored the reduced glutathion, and enhanced the superoxide dismutase activity. These results highlight the ability of *Aframomum melegueta* to ameliorate oxidative damage in the liver and the observed effects are associated with its antioxidant activities.

Keywords: *Aframomum melegueta,* ethanol, oxidative stress, antioxidants, hepatoprotective effects

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INTRODUCTION

The liver plays a pivotal role in the metabolism of toxic substances that enter the body. Alcohol is one of these toxic substances, which is ultimately broken down into simple end-products for easy elimination (Fernandez-Checa *et al*., 1997; Fernandez-Checa, 2003). However, certain byproducts generated during alcohol metabolism may be more toxic than alcohol itself and may contribute to the development of alcohol liver disease (Ashak *et al*., 1991). Alcohol is the major culprit of liver disease in Western countries and this arises from excessive ingestion of alcohol (Lieber, 2000). 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 (Cederbaum, 2001; Cederbaum *et al*., 2009).

Alcoholic liver diseases (ALD) have attracted the attention of researchers all over the world. Although the underlying mechanisms are still to be well understood, increasing evidence indicates an involvement of oxidative stress in the development of ALD (Zeng *et al*., 2008). Earlier studies have demonstrated that ethanol-induced liver injury is associated with enhanced lipid peroxidation, protein carbonyl formation, formation of the 1-hydroxyl ethyl radicals, formation of lipid radicals and decreased hepatic antioxidant defense capabilities, especially glutathione (GSH) (Dey & Cederbaum, 2006). 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 (McDonough, 2003; Wang *et al*., 2006; Srivastava & Shivanandappa, 2006; Liu *et al*., 2010).

Some natural compounds isolated from roots, bark, fruit and seeds have been used to prevent the oxidative challenges against the liver during alcohol metabolism. The protective effects of these natural compounds were believed to be associated with their antioxidant activity (Okwu, 2005; Choi *et al.,* 2006). *Aframomum melegueta* (Alligator pepper) (Zingiberaceae) is one of such plants having both medicinal and nutritive values, and popularly used as herbal remedy against a wide range of ailments, both in Nigeria and several other countries of the world (Agoha, 1974). Alligator pepper is widely used by many cultures in Nigeria for various purposes. It is served along with Kola nuts and alcoholic drinks to guests as entertainment. It is a common ingredient in pepper soup, a spicy delight in most parts of West Africa (Inegbenebor *et al*., 2009). The seed is commonly known as alligator pepper in Nigeria and has indigenous names as atare (Yoruba), osi-oji (Ibo), and citta (Hausa).

The present study is intended to explore the hepatoprotective effect of aqueous extract of *A. melegueta* when co-administered with ethanol by gavage.

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Abbreviations: ALT, alanine aminotransferases; A.M., *Aframomum melegueta;* AST, aspartate aminotransferases; b.w., body weight; GSH, reduced glutathione; GST, gluthathione-S-transferase; LPO, lipid peroxidation; MDA, malondialdehyde; SOD, superoxide dismutase; TG, triglyceride; ROS, reactive oxygen species

MATERIALS AND METHODS

Chemicals. Randox alanine aminotransferase (ALT), aspartate aminotransferase (AST), and triglyceride (TG) assay kits were purchased from ABJ Chemicals (Lagos, Nigeria). Adrenaline, thiobarbituric acid (TBA), Ellmans reagent (DTNB), glutathione (GSH) and bovine serum albumin (BSA) were purchased from Sigma Chemical (St Louis, MO, USA). All other chemicals were of the highest purity commercially available.

Animals. Twenty-four male Wistar rats were obtained from the Animal house of the Biochemistry Department, College of Medicine, University of Ibadan, weighing between 140 g and 170 g. The animals were allowed access to feed and water *ad libitum* for a period of seventeen days, for their acclimatization prior to the commencement of the experiment. The animals were kept in well ventilated cages at room temperature (28-30 °C), and under controlled light cycles (12 h light/12 h dark). All procedures were carried out in accordance with the conventional guidelines of the National Institutes of Health (Maryland, USA) for experimentation with animals.

Plant material. *Aframomum melegueta* fruits were purchased from Bodija market (Nigeria), identified and authenticated by the Botany Department, University of Ibadan, and a voucher was kept. One kilogram of seeds from air-dried fruits was pulverized into uniform powder using an electric blender (25–28 °C), packed in airtight bottles and stored untill required for extraction. Phytochemical screening of dry seeds of *A. melegueta* was carried out in the Department of Pharmacognosy (University of Ibadan, Ibadan, Nigeria). Standard method of Harbone (1973) was adopted.

Preparation and administration of the extract. Pulverized seed (600 g) was extracted with 1500 ml of distilled water by maceration for 72 hours. The aqueous extract was filtered and the filtrate was concentrated in a rotary evaporator to yield a yellowish brown extract. This was carefully scraped into a clean sample bottle and stored in a refrigerator at 4 °C for further use. Aqueous extracts of *A. melegueta* were used for the experiment. Twenty-four male Wistar rats were randomly distributed into four groups of six animals each. Group 1 served as the control and received corn oil as vehicle for *A. melegueta* administration. Group 2 received 45% ethanol only (4.8 g/kg). The third group received 45% ethanol (4.8 g/kg) and *A. melegueta* extract (100 mg/kg), while the fourth group received 45% ethanol (4.8 g/kg) and *A. melegueta* extract (200 mg/kg). Administration of the extract was by orall gavage using a cannula. *A. melegueta* extract was dissolved by gentle agitations with a stirring rod in corn oil, and 96% ethanol was diluted with distilled water to 45%. Animals received daily doses for 16 days and were observed daily for psychomotor changes and other signs of toxicity including death throughout the period of study.

Preparation of tissues for biochemical analyses and histological examination. Following the daily exposure for 16 days, the animals were sacrificed 24 hours after the last dose. Liver samples were quickly excised and washed in ice-cold 1.15% KCl solution, dried using filter paper and weighed. They were then homogenized in 4 volumes of 56 mM Tris/HCl buffer (pH 7.4) containing 1.15% potassium chloride and centrifuged at $10\,000 \times g$ for 15 min. The supernatant was collected and stored until needed for assays. Small pieces of liver sections were fixed in 10% formal saline for histological assessment which was carried out in the Department of Veterinary Anatomy (University of Ibadan, Ibadan, Nigeria).

Biochemical assays. Protein concentrations of the various samples were determined by means of the Biuret method as described by Gornal *et al.* (1949). Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation by following the method of Varshney and Kale (1990). The level of SOD activity was determined by the method of Misra and Fridovich (1972). The liver fraction was reacted with adrenaline solution and the rate of inhibition of adenochrome formation from the auto-oxidation of adrenaline was measured at 480 nm.

The levels of reduced glutathione (GSH) in the supernatant fraction of the liver homogenate were estimated using the method described by Beutler *et al.* (1963). Glutathione-*S*-transferase activity was determined according to Habig *et al.* (1974). Serum alanine and aspartate aminotransferases (ALT and AST) and serum triglyceride were quantified spectrophotometrically using Randox commercial assay kits.

Statistical analysis. All data were expressed as mean ± S.D. One-way analysis of variance (ANOVA) was used for the analysis of the biochemical indices. Differences were considered significant at $P < 0.05$.

RESULTS

Results of the phytochemical screening (carried out in the Department of Pharmacognosy, University of Ibadan, Ibadan) revealed the presence of alkaloids, phenols, flavonoids, saponins and tannins in the aqueous extract of *A. melegueta* seeds. At the end of the experiment, an increase in body weight was observed in all animal groups (Table 1). The mean body weight gain by group 2 treated with 45% ethanol was significantly higher than for group 4 treated with 45% ethanol and A.M. 200 mg/kg. Treatment with ethanol led to a significant increase in LPO in group 2 when compared with

Table 1. Effect of *Aframomum melegueta* on body weight and organ weight

	Body weight (g)		Weight gain (g)	Organ weight (g)	
Group	Initial	Final	(%) Increase	Liver	Relative liver weight (g)
Group 1	150.00 ± 0.00	167.00 ± 7.58	11.33	5.15 ± 0.24	3.08 ± 0.14
Group 2	140.00 ± 13.69[d]	158.60 ± 10.23[d]	13.28	5.20 ± 0.37	3.33 ± 0.32
Group 3	150.00 ± 17.67	169.60 ± 19.19	13.06	5.58 ± 0.51	3.33 ± 0.59
Group 4	170.00 ± 20.91[b]	183.60 ± 22.05[b]	8.00	5.56 ± 0.51	3.06 ± 0.56

Values shown are mean ± S.D. (n = 6). Mean differences are significant ($P<0.05$) when compared with: [a]control group, [b]group 2 (45% ethanol), [c]group 3 (45% ethanol + A.M. 100 mg/kg b.w.t.), [d]group 4 (45% ethanol + A.M. 200 mg/kg b.w.t.).

Table 2. Effect of *Aframomum melegueta* on hepatic MDA levels and hepatic antioxidant system

Group	MDA (nmol/mg protein)	GSH (µg/mg protein)	SOD (Units/mg protein)	GST (Units/mg protein)
Group 1	7.54 ± 0.27[bc]	18.40 ± 7.26	0.93 ± 0.40[bcd]	0.24 ± 0.04
Group 2	21.22 ± 9.72[ad]	13.20 ± 3.03[d]	0.26 ± 0.13[a]	0.22 ± 0.03
Group 3	17.57 ± 5.24[ad]	17.20 ± 1.09	0.37 ± 0.10[a]	0.22 ± 0.04
Group 4	7.65 ± 0.11[bc]	20.40 ± 5.89[b]	0.42 ± 0.23[a]	0.24 ± 0.04

Values shown are mean ±S.D. (n = 6). Mean differences are significant ($P < 0.05$) when compared with: [a]control group, [b]group 2 (45% ethanol), [c]group 3 (45% ethanol + A.M. 100 mg/kg b.w.t.), [d]group 4 (45% ethanol + A.M. 200 mg/kg b.w.t.). MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; GST, glutathione-*S*-transferase.

group 1 (control), as shown in hepatic MDA levels (Table 2). Co-administration of A.M. (100 mg/kg) reduced the hepatic MDA levels slightly while co-administration of A.M. (200 mg/kg) led to a significant reduction in hepatic MDA levels to almost the control values.

There was no significant decrease in the GSH level of group 2 administered 45% ethanol when compared with group 1 (Table 2). On the other hand, group 4 (co-administration of A.M. 200 mg/kg) showed restoration of the GSH level.

A noticeable reduction in the activity of SOD in group 2 was observed (Table 2) compared with group 1. Co-administration of A.M. (100 or 200 mg/kg) markedly enhanced SOD activities. There was no significant reduction in GST activity in the liver in group 2 treated with ethanol when compared with group 1. GST activity remained unchanged following co-administration of A.M. (100 or 200 mg/kg) when compared with group 1.

The activity of serum ALT in group 2 (Table 3) revealed a significant increase on ethanol administration when compared with group 1, while groups 3 and 4 showed significant reduction in the serum ALT activity when compared with group 2.

There was an insignificant increase in the level of serum AST in group 2 when compared with group 1. There was no significant reduction in the level of serum AST in the groups treated with ethanol and the different doses of *A. melegueta* when compared with the control, but the reduction was significant when group 2 and group 4 were compared (Table 3).

There was a significant increase in the level of serum triglyceride in group 2, group 3 and group 4 when compared with group 1, and a significant decrease in the level of serum triglyceride in the groups treated with different doses of *A. melegueta* (group 3 and 4) when compared with group 2 (Table 3).

Histological assessment of the liver revealed severe central venous and portal congestion in group 2 and marked central venous congestion and diffuse hydropic degeneration in group 3. No visible lesion was seen in group 1 while very mild diffuse hydropic degeneration was observed in group 4.

Table 3. Effect of *Aframomum melegueta* on serum AST, ALT and TG levels

Group	AST (UI/L)	ALT (UI/L)	TG (mmol/L)
Group 1	9.08 ± 0.73	51.00 ± 6.89[b]	3.00 ± 0.01[bcd]
Group 2	9.94 ± 0.98[d]	73.60 ± 12.13[ac]	3.40 ± 0.00[acd]
Group 3	9.04 ± 0.76	60.08 ± 11.67[b]	3.25 ± 0.00[abd]
Group 4	8.34 ± 0.23[b]	52.02 ± 5.44[b]	3.10 ± 0.00[abc]

Values shown are mean ±S.D. (n = 6). Mean differences are significant ($P<0.05$) when compared with: [a]control group, [b]group 2 (45% ethanol), [c]group 3 (45% ethanol + A.M. 100 mg/kg b.w.t.), [d]group 4 (45% ethanol + A.M. 200 mg/kg b.w.t.). AST, aspartate aminotransferases; ALT, alanine aminotransferases; TG, triglyceride

DISCUSSION

Alcohol consumption affects the liver and other organs and could contribute to the development of alcohol liver disease (Nordman *et al.*, 1992). In this study, ethanol administration significantly induced lipid peroxidation (LPO). This agrees with the findings of Mansouri *et al.*, (1997), Arteel *et al.* (1999) and Albano (2006). In the current study, the reduction we observed in the levels of LPO in groups 3 and 4 could be associated with the protective effect of high doses of *A. melegueta* on ethanol-induced toxicity in the liver and a contributing protective role of detoxifying enzymes which are more abundant in the liver than in other tissues (Autrup, 2000).

GSH plays an important role in the antioxidant effects, nutrient metabolism and regulation of cellular events (Wu *et al.*, 2004). In addition, GSH can also react with various electrophiles in a reaction catalyzed by glutathione-*S*-transferase to form mercapturates, (Zeng *et al.,* 2008). Our results document effect of ethanol on the hepatic antioxidants especially GSH, as indicated by LPO induction. Consequently, the decrease observed in the GSH level could be due to its utilization to counteract the effects of reactive oxygen species (ROS).

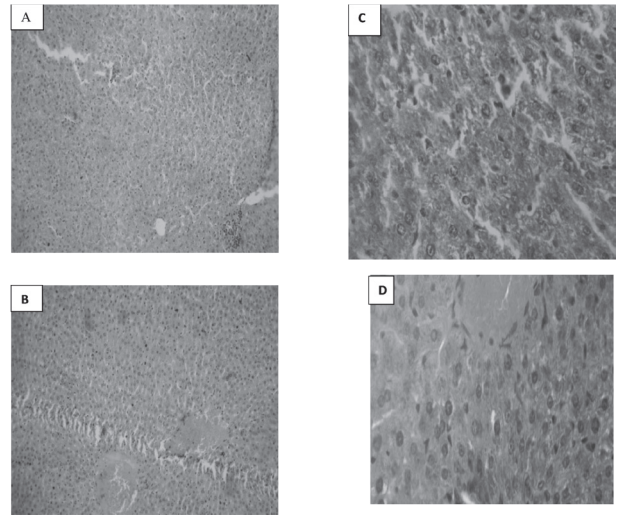

Figure 1. Effects of *Aframomum melegueta* on rat liver treated with ethanol
(A) Group 1: no visible lesions. **(B)** Group 2: severe central venous and portal congestion, portal fibroplasias. **(C)** Group 3: marked central venous congestion, diffuse hydropic degeneration. (D) Group 4: diffuse hydropic degeneration (very mild).

One main function of GST is to catalyze the biotransformation of xenobiotics, leading to detoxification (Hayes & Pulford, 1995). The marked reduction in the activity of SOD in rats administered 45% ethanol and the enhanced SOD activities when A.M. was co-administered were also in agreement with other studies (Das *et al.,* 2008; Adaramoye *et al.,* 2009). SOD catalyzes the clearance of superoxide anion radicals, preventing the formation of H_2O_2. A reduction in SOD activity can be attributed to increased production of H_2O_2 which prevents conversion to hydroxyl radicals thus protecting the visceral organs from oxidative damage (Albano, 2006). The restoration and enhancement of the GSH and SOD levels and activities may account for the protective effects of *A. melegueta* extract.

The high level of ALT and AST in the serum is an indication of the degree of damage to the liver caused by ethanol (Valentine *et al*., 1990; Bain, 2003; Kudo *et al*., 2009; Chen, 2010). The slight reduction in the level of ALT and AST activity in the groups co-administered A.M. at 200 mg/kg may be due to the antioxidant activity of A.M. (Arteel, 2003).

We also noticed that ethanol administration led to a significant increase in the level of serum triglyceride, which was attenuated by the different doses of A.M. This is consistent with the work of Adiels *et al.* (2008) and Karthikesan and Pari (2008). Triglyceride levels usually increase as body weight increases. The significant reduction in the serum level of triglyceride in groups 3 and 4 may be due to the smaller increase in body mass and or to a direct effect of secondary metabolites present in the plant.

In conclusion, our findings clearly demonstrate that administration of 45% ethanol at a dose of 4.8 g/kg to male rats depleted the hepatic antioxidant systems. The adverse effect of ethanol on the liver is due to the impairment of its antioxidant defense. Phenols and flavonoids are hepatoprotectives. Therefore, the observed hepatoprotective and antioxidant activity of A.M. could be attributed to the presence of phenols and flavonoids. Aqueous extract of A.M. can function as an effective free-radical scavenger, thereby preventing hepatic injury.

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