Hepatoprotective effect of *Piper guineense* aqueous extract against ethanol-induced toxicity in male rats

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**Summary**

**Objective:** Herbal medicinal products play an important role in the management of liver diseases for the lack of satisfactory liver protective drugs in allopathic medical practices. Searching for hepatoprotective drugs with high efficacy and safety is of great need. Our aim is to evaluate the hepatoprotective and antioxidant effect of aqueous extract of *Piper guineense* (P.G.) on ethanol induced toxicity in Wistar rats.

**Methods:** In order to assess the hepatoprotective effect of this extract in experimental animals, twenty-four Wistar male albino rats (weighing 150-170 g) were divided into four groups. Toxicity was induced by administering 45% ethanol (4.8 g/kg b.w) by oral gavage for 21 days. Serum triglyceride (TG) levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were monitored. Thiobarbituric acid reactive substances, reduced glutathione (GSH) levels, superoxide dismutase (SOD) and glutathione-S-transferase (GST) activities were determined in the liver.

**Results:** At the end of the experiment, chronic administration of ethanol resulted in enhanced lipid peroxidation (LPO) with depletion in the levels of GSH as well as reduction in the activities of SOD and GST. TG levels, ALT and AST activities were elevated. This was attenuated by the co-administration of the *P.guineense* extract by oral gavage (100 or 200 mg/kg b.w). Administration of the plant extract during ethanol exposure inhibited hepatic LPO and ameliorated SOD and GST activities as well as restoring GSH levels significantly.

**Conclusion:** From this study it can be concluded that aqueous extract of *P.guineense* possess some potent antioxidants which can ameliorate hepatic damage associated with chronic ethanol exposure in rat models.

**Key words:** Antioxidants; Ethanol; Hepatoprotective; Oxidative damage; *Piper guineense*; Rats

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**Received:** August 23, 2011 **Accepted:** October 14, 2011 **Published online:** November 24, 2011

**J Exp Integr Med 2012; 2:71-76**

DOI:10.5455/jeim.241111.or.016

**Introduction**

In recent years there have been remarkable developments in the prevention of diseases, especially with regards to the role of free radicals and antioxidants. Since oxidative stress may be involved in alcoholic liver disease, ethanol-induced oxidative stress appears to be one mechanism by which ethanol causes liver injury. 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 the involvement of oxidative stress in the development of ALD [1]. Almost all ingested alcohol is metabolized in the liver and excessive alcohol consumption may lead to acute and chronic liver disease [2, 3].

Earlier 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 decreased hepatic antioxidant defense capabilities, especially glutathione (GSH) [4]. Medicinal plants possessing natural antioxidants polyphenolics such as anthraquinones, flavonoids, aromatic acids, and tannins have been shown to have reactive oxygen species (ROS) scavenging and lipid peroxidation preventing effects [5, 6].

*Piper guineense* also known as African black pepper is one of such plants having both medicinal and nutritive values. *Piper guineense* is a climbing perennial plant of the family *Piperaceae* [7, 8]. It is used as spices and preservatives; it also has applications as insecticides and is used in herbal medicine and in the cosmetic industry [9-12]. The seed is commonly known as black pepper in Nigeria and has indigenous names as *Iyere* (Yoruba) and Uziza (Ibo). This study was designed to evaluate the hepatoprotective and antioxidant effect of aqueous extract of *P.guineense* on ethanol-induced toxicity in rat model.
Materials and methods

Animals

Twenty-four Wistar male albino rats (weighing 150-170 g) were obtained from the Animal House of the Biochemistry Department, College of Medicine, University of Ibadan. The animals were weight were allowed access to feed (obtained from Ladokun Feed Mill Nigeria Limited, Ibadan, Nigeria) 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, and under controlled light/dark cycles (12/12 h). All procedures were carried out in accordance with the conventional guidelines of the National Institutes of Health, Maryland, USA, for experimentation with animals.

Chemicals

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

Plant material

*Piper guineense* seeds were purchased from Bodija market in, Ibadan metropolis (Nigeria), identified and authenticated by the Botany Department, University of Ibadan. One kilogram of the seeds from air-dried seeds was pulverized into uniform powder using an electric blender (25-28°C) and packed in airtight bottles and stored until required for extraction. Phytochemical screening of dry seeds of *P. guineense* was carried out in the Department of Pharmacognosy, University of Ibadan. Standard method of Harborne [13] 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 (Buchi Rotavapor RE-3; Buchi Labortechnik AG, Flawil, Switzerland) 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 *P. guineense* were used for the experiment. Twenty-four male wistar rats were randomly distributed into four groups of six animals each. Group I served as the control and received corn oil as vehicle for *P. guineense* administration. Group II received 45% ethanol only (4.8 g/kg). The third group received 45% ethanol (4.8 g/kg) and 100 mg/kg *P. guineense* extract, while the fourth group received 45% ethanol (4.8 g/kg) and 200 mg/kg *P. guineense* extract. Administration of ethanol and the extract was by oral gavage using a cannula. *Piper guineense* 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 21 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 21 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% KCl, and then centrifuged at 10,000g for 15 minutes. The supernatant was collected and stored until needed for assays. Small pieces of liver sections were fixed in 10% formal saline. Sections were cut and stained with haematoxylin and eosin. The stained tissue sections were observed under a light microscope (x400 objective) for histological assessment, which was carried out in the Department of Veterinary Anatomy, University of Ibadan, Ibadan.

Biochemical assays

Protein concentrations of the various samples were determined by means of the Biuret method as described by Gornall *et al* [14]. Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation by following the method of Varshney and Kale [15]. The level of SOD activity was determined by the method of Misra and Fridovich [16]. 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* [17]. Glutathione-S-transferase (GST) activity was determined according to Habig *et al* [18]. Serum ALT and AST activites and TG levels were quantified spectrophotometrically using Randox commercial assay kit.
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. This was done using SPSS software (15.0). Differences were considered significant at p<0.05.

Results
Results of the phytochemical screening (carried out in the Department of Pharmacognosy, University of Ibadan), revealed the presence of alkaloids, flavonoids, saponins, tannins and glycosides in the aqueous extract of Piper guineense seeds. The aqueous extract of P.guineense did not show any sign or symptoms of toxicity and no mortality was recorded during the study. An increase in body weight was observed in all animals groups (Table 1). The effect of aqueous extract of P.guineense on serum transaminases and TG are summarized in Table 2.

There was a significant (p<0.05) increase in serum transaminases activities and TG level in ethanol intoxicated group when compared with the control. Co-administration of aqueous extract of P.guineense at a dose of 100 or 200 mg/kg significantly (p<0.05) decreased the elevated levels of the serum marker enzymes and TG.

Treatment with ethanol led to a significant (p<0.05) increase in the levels of the end products of lipid peroxidation (LPO) when compared with control group, as shown in Fig.1. Co-administration of P.guineense extract (100 or 200 mg/kg) reduced the hepatic MDA levels to almost normal.

The GSH levels, as well as SOD and GST activities in 45% ethanol treated rats were significantly (p<0.05) reduced compared to the control as shown in Figs.2, 3 and 4, respectively. Co-administration of P.guineense extract (100 or 200 mg/kg) to rats was found to markedly enhance GSH levels, as well as SOD and GST activities.

Histopathological examinations of the liver sections were carried out to further confirm the extent of the liver damage (Fig.5). However, no visible lesion was seen in the liver sections of the control group while there was severe central venous and portal congestion as well as portal fibroplasias in the liver section of 45% ethanol treated rats. Diffuse hydropic degeneration and cellular infiltration by mononuclear cells was observed in liver section of 45% ethanol + P.guineense extract 100 mg/kg treated rats while mild periportal hepatic necrosis and cellular infiltration was seen in 45% ethanol + P.guineense extract 200 mg/kg treated rats liver section.

Table 1. Effect of Piper guineense on body weight and organ weight (mean±S.D., n=6 for each group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Weight gain (% increase)</th>
<th>Liver weight (g)</th>
<th>Relative liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>150±10^a</td>
<td>167.00±7.58</td>
<td>11.33</td>
<td>5.51±0.24^c,d</td>
<td>3.08±0.14</td>
</tr>
<tr>
<td>45% ethanol (4.8g/kg)</td>
<td>140±13.69^ed</td>
<td>158.6±10.23^ed</td>
<td>13.28</td>
<td>5.20±0.37^ed</td>
<td>3.33±0.32</td>
</tr>
<tr>
<td>45% ethanol + P.guineense 100 mg/kg</td>
<td>165±13.69^ab</td>
<td>178.20±5.89^b</td>
<td>5.87±0.57^ab</td>
<td>9.54</td>
<td>3.29±0.36</td>
</tr>
<tr>
<td>45% ethanol + P.guineense 200 mg/kg</td>
<td>155±11.18^b</td>
<td>173.20±9.31^b</td>
<td>5.96±0.47^ab</td>
<td>11.74</td>
<td>3.45±0.42</td>
</tr>
</tbody>
</table>

Mean differences are significant (p<0.05) when compared with: *control group, *45% ethanol, *45% ethanol + P.guineense 100 mg/kg, *45% ethanol + P.guineense 200 mg/kg
Table 2. Effect of Piper guineense on serum transaminases activities and triglyceride levels (mean±S.D., n=6 for each group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (UI/L)</th>
<th>ALT (UI/L)</th>
<th>TG (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.08±0.73b</td>
<td>51.00±6.89bc</td>
<td>3.00±0.01bcd</td>
</tr>
<tr>
<td>45% ethanol (4.8g/kg)</td>
<td>9.94±0.98ad</td>
<td>73.60±12.13ad</td>
<td>3.40±0.06acd</td>
</tr>
<tr>
<td>45% ethanol + P.guineense 100 mg/kg</td>
<td>9.35±0.11</td>
<td>66.20±13.06a</td>
<td>3.29±0.00abcd</td>
</tr>
<tr>
<td>45% ethanol + P.guineense 200 mg/kg</td>
<td>8.89±0.25b</td>
<td>62.70±6.63b</td>
<td>3.17±0.01abc</td>
</tr>
</tbody>
</table>

Mean differences are significant (p<0.05) when compared with: *control group, 45% ethanol, 45% ethanol + P.guineense 100 mg/kg, 45% ethanol + P.guineense 200 mg/kg (AST, aspartate aminotransferase; ALT, alanine aminotransferase; TG, triglyceride).

**Discussion**

Consumption of alcohol affects the liver and other organs and could contribute to the development of alcohol liver disease [19]. The elevation observed in serum levels of ALT and AST is an indication of the degree of damages to the liver caused by the ethanol [20, 21]. Clinically, measurements of serum ALT, AST and γ-glutamyltransferase (GGT) are widely used as markers in evaluating the degree of liver injury. ALT is the more specific measure of alcohol-induced liver injury because it is found predominantly in the liver, whereas AST is found in several organs, including the liver, heart, muscle, kidney, and brain [22]. The reduction in the levels of ALT and AST by P.guineense extract at a dose of 100 or 200 mg/kg b.w, therefore suggests that the extract is not toxic or damaging to the integrity of the liver but possibly hepatoprotective.

Worthy of note is the fact that ethanol administration led to a significant increase in the...
level of serum TG, which was markedly reduced by the different doses of *P. guineense* extract. Our finding is in agreement with the work of Adiels *et al.* [23] as well as Karthikesan and Pari [24] suggesting that *P. guineense* has a greatest potential for protection against atherosclerosis and its accompanying risk of cardiovascular diseases.

The results of the present study shows an elevated MDA levels in liver of rats treated with ethanol, this suggests enhanced lipid peroxidation. This could be as a result of the overwhelming effect of the excessive free radicals generated by ethanol on the hepatic antioxidants. Lipid peroxidation causes impairment of biological membrane functions, *e.g.*, decreases fluidity, inactivates membrane bound enzymes receptors etc [25]. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues. When this process occurs in biological membrane, obviously alteration of structural, organization and enzyme function may result. The reduction we observed in the levels of LPO when *P. guineense* extract at a dose of 100 or 200 mg/kg was administered could be associated with the protective effect of *P. guineense*. This may be as a result of contributing protective role of all other detoxifying enzymes which are mostly abundant in the liver of animals than other tissues [26].

Superoxide dismutase, catalase (CAT) and GST constitute a mutually supportive team of defense against ROS. Yamamoto and Yamashita [27] reported that ROS affects the antioxidant defense mechanisms, by reducing the intracellular concentration of GSH, decreasing the activities of SOD and the detoxification system produced by GST. GSH and SOD are important endogenous antioxidants. The decrease in GSH level could be due to increased GSH consumption as it participates in the detoxification system for the metabolism of ethanol. While on the other hand the reduction observed in SOD activity may be as a result of exhaustion of SOD in bid to scavenge the excessive ROS produced as a result of ethanol toxicity. SOD converts superoxide radical to hydrogen peroxide that is subsequently converted to water by CAT and GPX. This is similar to the findings of Manna *et al.* [28] and Palanivel *et al.* [29].

Administration of the varying doses of the plant extract resulted in about two fold increase in the activities of GSH and SOD; the increase observed could be as a result of bioactive constituents of the extracts mainly alkaloids and flavonoids. It was

![Figure 5](image-url)
also observed that ethanol treatment led to a significant decrease in the activity of GST, this could be associated with its role in the biotransformation and detoxification of ethanol [30]. We noticed that co-administration of *P. guineense* extract at a dose of 100 or 200 mg/kg resulted in an insignificant increase. The exact reason for this is not clearly known. Histological changes were related to the results obtained.

In conclusion, the results presented above showed that aqueous extract of *Piper guineense* possess some potent antioxidants which can ameliorate hepatic damage caused by ethanol exposure in rat models. It also confirms the ability of *P. guineense* to protect against ethanol-induced toxicity by enhancing the antioxidant defence systems, and attenuating lipid peroxidation in the liver of rats.

Acknowledgements

The authors declare that they have no conflict of interest.

References


