Ethanol-induced hepatotoxicity in male wistar rats: Effects of aqueous leaf extract of *Ocimum gratissimum*

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Abstract

*Ocimum gratissimum* leaf is commonly consumed as a spice and sometimes used in folklore medicine in Nigeria and other parts of the world. It has been reported to be rich in natural antioxidants such as flavonoids. The present study sought to evaluate the protective effects of its orally administered aqueous extract (0.2g/kg bwt/day and 0.4g/kg bwt/day for 35 days) on ethanol–induced oxidative stress in male wistar rats. The activities of ALT, AST, GGT and ALP in the serum and liver homogenate were determined. The activities of GST, CAT and SOD as well as the concentrations of MDA and GSH formed due to oxidative stress were also evaluated. Relative to the control group, treatment with ethanol significantly (P<0.05) raised the serum activities of ALT, AST, ALP, GGT and simultaneously lowered the hepatic activities of these enzymes. The level of MDA and GST in the liver were also significantly increased while SOD and CAT activities as well as reduced GSH concentration were markedly (P<0.05) decreased following administration of ethanol. However, the extract especially at a dose of 0.4g/kg of body weight significantly reversed the activity/concentration (near normal) of the analyzed enzymes and molecules. The data of the study suggest that the investigated extract protected the rats against ethanol-induced hepatotoxicity.

Keywords: *Ocimum gratissimum*, ethanol, rats, hepatic, enzyme markers, oxidative stress.

INTRODUCTION

Ethanol is the psychoactive compound present in alcoholic drinks. The etiology of some oxidative stress -based pathological conditions in the liver has implicated excessive alcohol consumption. More than ever before, there is an upsurge in alcohol abuse and as a result, alcohol-related disorders are becoming increasingly important causes of morbidity and mortality, globally (Rukumani *et al.*, 2004). Many research reports have linked chronic alcohol consumption and variety of pathological conditions varying from simple intoxication to severe life-threatening pathological states. (Tsukamoto and Lu, 2001; McDonough, 2003; Tuma, 2002; Lieber, 2003; Molina *et al.*, 2002).
The liver is particularly susceptible to alcohol–related injury such as fatty liver, hepatitis, and cirrhosis because it is the primary site of alcohol metabolism. Alcohol-induced hepatotoxicity has been observed to develop mainly through excessive generation of free radicals and reactive oxygen species, as well as impaired anti-oxidant defense mechanism; conditions which result in oxidative stress with attendant health problems (Wu and Cederbrum, 2003). Excessive alcohol consumption also has a negative effect on the nutritional status of the alcoholics, interfering with the digestion, absorption and utilization of essential nutrients such as vitamins, minerals and proteins. (Gruchow, et al., 1985; Liebe, 2003).

Conversely, *Ocimum gratissimum* is rich in polyphenolic compounds with antioxidant properties, thus, it possesses the ability to scavenge free radicals and reactive oxygen species (ROS). The plant is an edible vegetable popularly referred to as scent-leaf because of its characteristic aromatic smell. It belongs to the family of *Labiatae*. It is widely distributed across Nigeria and some other parts of the world. In the Edo and Igbo speaking areas, the plant is known as ebamwonkho and nchu-anwu respectively. In the Northern part, it is generally called Dai doya togida while it is commonly referred to as efirrin by the Yoruba speaking people.

Preparation and administration of ethanol

5g per kg body weight of 20% v/v ethanol solution was used as chronic dose in this experiment. 20g absolute ethanol was dissolved in distilled water and made up to 100ml. 4.25ml of the solution was daily administered for four weeks to each rat treated with ethanol.

Animal management and Administration

Fifty male albino rats of Wistar strain were used for the experiment. They were purchased from the Institute for Advanced Medical Research and Training (IMRAT), at the University College Hospital (UCH), Ibadan. The animals were handled humanely, kept in a plastic suspended cage placed in a well ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were provided with rat pellets, served water *ad libitum* and subjected to natural photoperiod of 12h light and 12h dark cycle. The rats attained a body weight range of 166g-170g before being used for this study. The animals were randomly assigned into five (5) groups of ten (10) rats each.

Group A (Control)

Rats fed with normal laboratory chow and with water *ad libitum*.

Group B (Og group)

Rats treated orally with *Ocimum gratissimum* (Og) extract only, 200mg /kg body weight (1.0ml extract solution / 170g bwt) daily for five weeks.

Group C (ET group)

Rats treated orally with ethanol only 5g/kg body weight (4.25ml ethanol solution/170g bwt) daily for four weeks.

Group D (CET group)

Rats treated orally with ethanol and *Ocimum gratissimum* extract combination (5g/kg ethanol + 200mg Og/kg body weight / week) daily for five weeks.

The purpose of the present study was to investigate the hepatoprotective effects of the plant leaf extract in rats treated with chronic alcohol.

**MATERIALS AND METHODS**

Preparation and administration of the plant material

Fresh *Ocimum gratissimum* leaves were collected from Ashi Community, Ibadan. The botanical identification was confirmed at the Herbarium of the Department of Botany, University of Ibadan.

The leaves were freed of extraneous materials, air dried at room temperature and ground into a uniform powdery form using a milling machine. The powdered leaf was macerated in distilled water and extracted twice, on each occasion with 2.5L distilled water at room temperature for 48h, with occasional shaking. The obtained distilled water extracts were concentrated under reduced pressure at 60±1°C in a water bath. Drying and solvent elimination finally gave a light brown extract. This crude aqueous extract was used, without further purification. 3.5 grams of the extract were dissolved in distilled water and made up to 100ml and was stored at 4°C until used. The volume of the extract solution containing the required amount of the extract was appropriately administered daily for five weeks to each rat treated with the extract.

**Preparation and administration of ethanol**

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**Preparation and administration of ethanol**

5g per kg body weight of 20% v/v ethanol solution was used as chronic dose in this experiment. 20g absolute ethanol was dissolved in distilled water and made up to 100ml. 4.25ml of the solution was daily administered for four weeks to each rat treated with ethanol.
Group D (Og & ET group)

Rats treated orally with ethanol, 5g/kg bwt and O. gratissimum extract, 200mg/kg bwt (1.0ml extract solution/170g bwt) daily for four and five weeks respectively.

Group E (Og, Og & ET group)

Rats treated orally with ethanol 5g/kg bwt, and O. gratissimum extract, 400mg/kg bwt (2.0ml extract solution/170g bwt) daily for four and five weeks respectively.

Experimental groups B, D, and E were pretreated with O. gratissimum aqueous extract for a period of one week. Treatment with extract continued for four more weeks during which groups D and E were co-treated with ethanol (20% v/v). At the end of the administration, the animals were fasted for 12 hours and sacrificed. All animal experiments were carried out without anesthesia during the study.

Tissue Preparation for Biochemical Analysis

The animals (Control and ethanol-treated) were fasted overnight, weighed and sacrificed by cervical dislocation 12h after the last treatment. Blood sample was collected from the retro orbital sinus of the eye by ocular puncture into non-heparinized bottles for serum enzymes analyses, using standard assay kits (Randox Lab Ltd. UK.), and the target organ (liver) was quickly excised from each rat. Each organ was separately washed in ice-cold 1.15% KCl solution, blotted and weighed. Each organ from different rats was separately homogenized in a volume of the homogenizing buffer (ice-cold Tris-HCl buffer 0.1M, pH 7.4) four times its weight, using a potter Elvehjem type homogenizer. The resulting homogenate in each case was centrifuged at 10,000g for 30 minutes in a Beckman L5-50B ultra centrifuge with a 220.78 V02 rotor at 4°C. The resultant supernatant was collected and used for different biochemical analyses. Storage was done under 0°C to 4°C to preserve enzyme activity.

Enzyme analysis

The activity of the enzyme alanine amino transferase (ALT) formerly known as glutamate pyruvate transaminase (GPT) and aspartate amino transferase (AST) formerly glutathione-oxaloacetate transaminase (GOT) was measured as described by Reitman and Frankel (1957), using transaminase kits from Randox laboratories (USA), along with freshly prepared 0.4M Sodium hydroxide solution in the case of ALT.

Gamma glutamyl transferase activity was estimated as described by Szasz and Persyn (1974) using Gamma-Glutamyl Transferase kit from Biolabo, France, and ALP was estimated by the method of Englehardt et al., (1970). The activity of Super oxide dismutase (SOD) in liver homogenate was estimated by the method of Misra and Fridovich (1972). The method of Sinha (1971) was used for the estimation of Catalase activity. Glutathione-transferase activity was estimated spectrophotometrically at 37°C according to the procedure of Habig et al., (1974).

Biochemical analysis

The estimation of the reduced glutathione (GSH) was done using the method of Jollow et al. (1974). Lipid peroxidation in liver homogenates was estimated spectrophotometrically using thiobarbituric acid-reactive substances (TBARS) method as described by Varshney and Kale (1990).

Statistical Analysis

Statistical analysis was carried out using the one-way analysis of variance (ANOVA), followed by student t-test. The level of significance was set at p<0.05. The results are presented as the mean ± S.D of ten analyses.

RESULTS AND DISCUSSION

Table 1 shows the serum and hepatic activities of ALT, AST, ALP and GGT in treated and non-treated rats. Ethanol treated rats showed a significant (P<0.05) rise in serum activities of ALT, AST, GGT and ALP and a concomitant reduction in the hepatic activities of these enzymes, relative to the non-treated rats.

The result is consistent with the findings of Uzun et al. (2005) who reported a significant increase in the activity of serum ALT and AST in rats, following ethanol administration. The respective rise and decrease in the serum and hepatic activities of these enzymes may be attributed to damaged structural integrity of the liver, which results in the leakage of these enzymes from the...
cytosol into the blood stream. This observation agrees with the report of Vermaulen et al. (1992) which stated that ALT, AST, GGT and ALP are normally located in the cytoplasm and released into circulation after cellular damage.

Co-administration of the extract with ethanol comparatively and markedly (P<0.05) reduced the activities of ALT, AST, ALP and GGT in the serum. Treatment with extract also resulted in a significant (P<0.05) increase in the hepatic activities of these enzymes in rats when compared to those exposed to ethanol alone (Table 1). These results suggest that O.gratissimum extract significantly inhibits liver derangement induced by ethanol. The investigated plant is rich in polyphenolic compounds such as flavonoids as part of its secondary metabolites (Ighodaro et al., 2009). This possibly accounts for its anti-oxidant property which enabled it to protect the liver against the disastrous effects of free radicals and reactive oxygen species (ROS). Oxidative stress results from a disturbance in the balance between generated oxidants and anti-oxidants in favour of the oxidants. This is often caused by an increase in the generation of reactive oxygen species (ROS) and a decrease in the activity of anti-oxidant system (Postmal et al., 1996. According to Albano et al. (1998), Wu and Cederbaum (2003), chronic alcohol consumption does not only activate free radical generation, but also alters the levels of both enzymatic and non-enzymatic endogenous antioxidant systems. This results in oxidative stress with cascade of effects, thus, affecting both functional and structural integrity of cell and organelle membranes (De level et al., 1996).

In this study the pathological effect of ethanol metabolism exemplified by decrease in reduced glutathione (GSH) level (Table 2), SOD and CAT activities (Figure 1) and concomitant increase in GST

### Table 1. Activities of enzyme markers of liver function in treated and non treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT IU/mg protein Serum</th>
<th>AST IU/mg protein Serum</th>
<th>ALP (U/L) Serum</th>
<th>GGT IU/mg protein Serum</th>
<th>ALT IU/mg protein Hepatic</th>
<th>AST IU/mg protein Hepatic</th>
<th>ALP (U/L) Hepatic</th>
<th>GGT IU/mg protein Hepatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>14.01 ± 1.01</td>
<td>23.51 ± 1.64</td>
<td>18.66 ± 1.28</td>
<td>28.05 ± 1.45</td>
<td>131.19 ± 4.02</td>
<td>167.44 ± 2.02</td>
<td>18.27 ± 1.28</td>
<td>95.09 ± 2.02</td>
</tr>
<tr>
<td>B (Og)</td>
<td>13.76 ± 0.32</td>
<td>22.85 ± 0.64</td>
<td>18.27 ± 1.28</td>
<td>95.09 ± 2.02</td>
<td>173.71 ± 3.02</td>
<td>177.31 ± 2.02</td>
<td>17.21 ± 1.25</td>
<td>73.95 ± 2.02</td>
</tr>
<tr>
<td>C (ET)</td>
<td>26.32 ± 0.19</td>
<td>48.39 ± 0.72</td>
<td>33.28 ± 0.15</td>
<td>79.56 ± 1.03</td>
<td>398.21 ± 3.02</td>
<td>418.21 ± 2.02</td>
<td>18.66 ± 1.28</td>
<td>95.09 ± 2.02</td>
</tr>
<tr>
<td>D (ET &amp; Og)</td>
<td>19.23 ± 0.45</td>
<td>31.43 ± 0.85</td>
<td>27.56 ± 0.21</td>
<td>119.34 ± 2.02</td>
<td>335.67 ± 3.02</td>
<td>335.67 ± 2.02</td>
<td>18.27 ± 1.28</td>
<td>95.09 ± 2.02</td>
</tr>
<tr>
<td>E (ET, Og &amp; Og)</td>
<td>14.68 ± 0.67</td>
<td>24.66 ± 0.87</td>
<td>21.98 ± 0.31</td>
<td>149.55 ± 1.02</td>
<td>402.51 ± 3.02</td>
<td>165.07 ± 3.02</td>
<td>18.27 ± 1.28</td>
<td>95.09 ± 2.02</td>
</tr>
</tbody>
</table>

P<0.05 = Significance. *=compared to control. **= compared to ET. a*= compared to ET & Og

### Table 2. Levels of oxidative-damage related parameters in treated & non-treated rats.

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>MDA (µmol/g tissue)</th>
<th>GSH (µg/g tissue)</th>
<th>GST (µg/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (control)</td>
<td>51.36 ± 0.09</td>
<td>12.38 ± 0.83</td>
<td>3.1 ± 0.43</td>
</tr>
<tr>
<td>B (Og only)</td>
<td>33.87 ± 2.02</td>
<td>19.23 ± 0.85</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>C (ET only)</td>
<td>107.39 ± 12.11**</td>
<td>119.34 ± 5.46**</td>
<td>8.6 ± 2.45**</td>
</tr>
<tr>
<td>D (ET &amp; Og)</td>
<td>52.12 ± 3.44**</td>
<td>8.56 ± 2.45**</td>
<td>5.4 ± 3.8**</td>
</tr>
<tr>
<td>E (ET, Og &amp; Et)</td>
<td>43.31 ± 5.27 a*</td>
<td>13.09 ± 2.22 a*</td>
<td>3.21 ± 0.20 a*</td>
</tr>
</tbody>
</table>

mean ± S.D for ten animals per group P<0.05 = Significance. *=compared to control. **= compared to ET. a*=compared to cET & Og
activity (Table 2) have been well observed. The enzymatic antioxidant defense system, which includes SODs and CATs, helps protect cells from oxidative injuries (Wu and Cederbaum, 2003). SOD catalyzes the rapid removal of superoxide radicals generating hydrogen peroxide which is eliminated by catalase (Wu and Cederbaum, 2003). These enzymes are present in the peroxisomes of nearly all aerobic cells. CAT protects the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals (Nehru and Anand, 2005) (Figure 2).
In the present study, ethanol treatment induced a significant (P<0.05) decrease in Reduced GSH content and SOD and CAT activities in the liver of rats when compared to the control group. These changes were markedly reversed by treatment with *O. gratissimum* extract. The reduction in the activities of these antioxidant enzymes may be due to the inhibition of their synthesis by some reactive molecules generated during ethanol metabolism. It could also be as a result of oxidation of the enzymatic proteins by the generated reactive oxygen species. GSH plays a significant role in both scavenging reactive oxygen species (ROS) and in the detoxification of xenobiotics (Sen, 1997). The decrease of this endogenous antioxidant is obviously connected with ethanol-induced oxidative stress, which is characterized by the generation of toxic acetaldehyde and other reactive molecules in the cell. The obtained result agrees with the findings of Hussan et al. (2001) and Molina et al. (2002) who reported that chronic ethanol treatment caused a significant reduction in hepatic GSH level. The observed increase in the reduced GSH level in rats co-treated with *O. gratissimum* extract and ethanol is likely due to the combined protective effects of the extract and the endogenous GSH. It may have also resulted from the induction of glutathione reductase which plays a critical role in the reduction of oxidized glutathione to reduced glutathione at the expense of NADPH and GSH- GSSG cycle in the cell.

Cells amplify antioxidant enzyme activities to counter sudden upsurge in oxygen metabolites, as to maintain the integrity of cellular membranes which is critical for normal cell function. Glutathione- s- transferase (GST) is a very important enzyme that plays a crucial role in the detoxification and metabolism of many foreign and endobiotic compounds. (Ji et al., 1992). Increase in GST activity is likely a defensive response to detoxify the toxic metabolites produced in the course of ethanol metabolism. When compared with rats treated with ethanol alone, co-treatment of rats with *O. gratissimum* extract and ethanol significantly (P<0.05) reduced the hepatic GST activity. The reduction in the hepatic GST activity may be attributed to the antioxidant properties associated with *O. gratissimum* extract which enabled it to protect the liver against the dilapidating effect of ethanol. Lipid peroxidation, an index of oxidative stress in vivo is often characterized by high concentration of malondialdehyde (MDA) (Arouma, 1998). A number of systems that generate reactive aldehydic species and reactive oxygen species are activated by chronic consumption of alcohol (Maher, 1997). This is consistent with the findings in this study which showed a significant increase (P<0.05) in hepatic malondialdehyde concentration by 109.1% in rats treated with ethanol relative to control. Alcohol metabolism which occurs primarily in the liver is characterized with the formation of free radicals and reactive oxygen species. As such, the observed high level of MDA in the liver could be adduced to the generation of free radicals resulting in the peroxidation of membrane lipids. Moreover, the main pathway for alcohol metabolism involves the enzyme alcohol dehydrogenase (ADH) (Maher, 1997; Pronkro et al., 2001). ADH metabolizes alcohol into toxic acetaldehyde, whose interaction with cell proteins and lipids can result in free radical generation and hepatocellular damage. Conversely, co-treatment of 0.2g/kg bwt/day and 0.4g/kg bwt/day of *O. gratissimum* extract with ethanol caused a marked (P<0.05) reduction in hepatic MDA level by 51.5% and 59.7% respectively, when compared with rats treated with ethanol alone (table 2). According to the reports of Kumar and Kutan, (2004), Kassuya et al., (2005) and Khatoon et al., (2005), the arrays of antioxidant phytochemicals present in a plant extract are responsible for the decrease in the level of lipid peroxidation (LPO). The antioxidant molecules in Ocimum gratissimum are likely responsible for the free radical scavenging activity exhibited by the extract in this study.

Histopathological examination of the liver section of the rats in the ethanol-treated group revealed an intense distortion of the hepatic architecture. The hepatic cells, intralobular veins and the endothelium were found to be damaged in the ethanol treated rats whereas negligible damage was seen in rats co-treated with the extract. The group treated with higher dose of the extract showed signs of protection against toxicity evident from reduced fatty degeneration and necrosis. The signs of hepatoprotection were not evident in the groups treated with lower dose of the extract. From the histological results, it is obvious that physiologic recovery preceded histological changes in the liver tissues.

**CONCLUSION**

On the basis of the available data in this report, it can be suggested that Ocimum gratissimum leaf extract elicit protection against ethanol-induced hepatic and oxidative damage in rats possibly by acting as an in vivo free radical scavenger or through induction of antioxidant enzymes, drug detoxifying enzymes, and prevention of excessive stimulation of lipid peroxidation.

The livers of ethanol treated rats showed massive fatty
changes, necrosis, and broad infiltration of the lymphocytes. The histological architecture of liver sections of the rats treated with the extract showed more or less normal patterns, with a mild degree of fatty change, necrosis and lymphocyte infiltration.

REFERENCES