Preventive and protective effects of wild basil in ethanol-induced liver toxicity in rats

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Accepted: 9 October 2006

ABSTRACT

In the present study, preventive and protective effects of *Ocimum gratissimum* in ethanol-induced hepatotoxicity are assessed in albino rats. A methanol extract of *O. gratissimum* leaves is prepared, with a yield of 3.5% (w/w) of the dry weight of leaves. Graded doses of the extract (10, 20, 40 and 80 mg/kg body weight), together with ethanol (5 gm/kg body weight) are administered orally to experimental groups for 30 days. Normal control rats receive distilled water only, while rats in an alcohol control group (AC) receive ethanol only for 30 days. *O. gratissimum* reduced the level of thiobarbituric acid reactive substance in all experimental groups (E1–E4). Alanine transaminase and aspartate transaminase levels fell in all experimental groups (E1–E4), but this reduction was significant only in groups E3 and E4 ($P<0.05$), indicating inhibition of lipid peroxidation by free radicals generated after ethanol metabolism. Levels of antioxidants also increased. Ascorbic acid and glutathione levels increased in all experimental groups (E1–E4; $P<0.05$ and $P<0.01$, respectively). A significant increase in catalase ($P<0.05$) was noted only in group E4, although an upward trend was noted in all experimental groups. This study shows that *O. gratissimum* prevents free radical damage to the liver and thus protects the organ from oxidative stress.

KEY WORDS: Alanine transaminase. Ascorbic acid. Aspartate aminotransferases. Catalase. Ethanol. Glutathione. Ocimum. Thiobarbituric acid.

Introduction

All energy-producing consumables undergo oxidation to generate energy. Oxidative processes produce reactive oxygen species (ROS), which cause oxidative changes in lipid, protein and DNA. Oxidative changes in lipid culminate into cell damage.[1] The body's antioxidant system neutralises these free radicals and thus provides protection against oxidative stress.

In certain circumstances, oxidation of some consumable items (e.g., ethanol) generates free radicals at a faster rate, and the antioxidant system fails to cope with severe oxidative stress.[2] In the liver, ethanol is oxidised to acetaldehyde and then to acetate.[3]

These processes are accompanied by generation of ethyl and hydroxyethyl radicals. Ethanol-inducible cytochrome CYP2E1 has high rate of NADPH oxidase activity, which leads to the rapid production of superoxide anions and hydrogen peroxide.[4] Thus, alcohol-induced hepatotoxicity is linked to oxidative stress.

Acetaldehyde also reacts with macromolecules of hepatic cells and causes liver damage.[5] Therefore, inhibition of free radical generation is important in providing protection against hepatic damage.

Several plants (e.g., *Ocimum sanctum*[6]) are known to possess antioxidant properties and are used to cure liver disorders in the Ayurvedic system of medicine. *O. gratissimum*, commonly known as wild basil, belongs to family Lamiaceae. In Africa the plant is used for the treatment of nasal congestion, cough and abdominal pain.[7]

The gastrointestinal cytoprotective potential of *O. suave* has been reported by Tan *et al.*,[8] and it also has a hypoglycaemic effect.[9] *O. sanctum* is also reported to have antioxidant properties.[10]

As *O. sanctum* and *O. gratissimum* belong to the same genus, it is proposed that *O. gratissimum* may also exhibit antioxidant properties. Hence, the present study aims to establish the antioxidant properties of the plant in terms of its protective effect against alcohol-induced liver damage.

Materials and methods

Plant material and preparation of extract

The plant material was collected around Gaborone, Botswana. It was identified taxonomically by Dr. M. P. Setshogo at the University of Botswana Herbarium (UCBA) as *O. gratissimum* L subsp. *gratissimum* var. *gratissimum*.

The leaves of the plant were harvested, dried and then crushed to a powder. The powder (300 g) was soaked with 1 L 70% methanol for three days. The plant extract was filtered, distilled and evaporated in a Buchi-type rotary vaporiser under reduced pressure to obtain the solvent-free extract.

The yield was 3.5% (w/w) of the powdered plant material. The extract was then fractionated by a liquid-liquid partitioning method with n-hexane, ethyl acetate and n-butanol. Thus, the methanol extract was separated into four subtypes (i.e., n-hexane extract, n-butanol extract, ethyl acetate extract and residual water extract). The present study was performed only on the methanol extract.

Chemicals

All chemicals used were of analytical grade and obtained from Sigma.

Animals

Male albino Wistar rats (200–250 g) were used in all experiments. Animals had free access to water and were fed a commercial diet (Nola Food, South Africa).

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Experimental design

Thirty rats were used in the experiments, divided into six groups of five. The normal control (NC) group received only distilled water. The alcohol control (AC) group received only ethanol. Group E1 received ethanol and plant extract (20 mg/kg body weight). Group E2 received ethanol and plant extract (40 mg/kg body weight). Group E3 received ethanol and plant extract (60 mg/kg body weight). Group E4 received ethanol and plant extract (80 mg/kg body weight).

The experiment was carried out over 30 days. Ethanol was given at 5 g/kg body weight to the AC group and all the experimental groups (E1–E4). At the end of the experiment, rats were bled to assay thiobarbituric acid reactive substance (TBARS), alanine transaminase (ALT) and aspartate transaminase (AST), reduced glutathione (GSH), catalase and ascorbic acid.

Student's *t* test was used for all statistical comparisons.

Biochemical tests

Lipid peroxidation in plasma was estimated in terms of thiobarbituric acid reactive substances by the method of Shushmakumari *et al*.,[11] with slight modification. Briefly, 0.1 mL plasma was treated with 2 mL TCA/TBA/HCL (0.37% TBA, 0.25N HCl, 15% TCA; equal parts) and incubated in boiling water for 10 min. The mixture was cooled, mixed with 2 mL freshly prepared 1N NaOH, and the absorbance was measured at 535 nm.

Alanine transaminase and AST were estimated using a kit from Sigma (Lot No. 079H6077 [ALT] and Lot No. 121k6123 [AST]), following the manufacturer's instructions.

Reduced glutathione was measured by the method of Ellman.[12] Briefly, 0.25 mL plasma was mixed with 0.5 mL precipitating buffer (1.67 g metaphosphoric acid, 0.2 g EDTA, 30 g sodium chloride, dissolved in 100 mL double-distilled water) and centrifuged. The supernatant was collected and mixed with 2.5 mL 0.3 mol/L phosphate buffer. Colour was developed by adding 100 µL 0.01% 5,5-dithiobis 2-nitrobenzoic acid (DTNB), read at 412 nm and expressed as mg/dL plasma

Ascorbic acid was assayed in plasma by the method of Roe,[13] with slight modification. Briefly, 1.5 mL 4% TCA was added to 0.5 mL plasma, and protein was precipitated by centrifugation at 3000 rpm for 20 min. Estimation of ascorbic acid content involved mixing 1 mL supernatant with 0.5 mL DNPH reagent (2 g dinitrophynyl hydrazine, 250 g thiourea, 30 mg $CuSO_4.5H_2O$ in 100 mL 9N sulphuric acid) and incubating for 3 h at 37°C. The colour was developed by adding 2.5 mL 85% sulphuric acid. Absorbance was recorded at 540 nm after 30 min.

Catalase was assayed by the method of Hans Bisswanger.[14] Briefly, 0.2 mL plasma was added to 0.98 mL H_2O_2 solution (10 mmol/L), and a decrease in the absorbance at 240 nm was followed. Catalase activity was calculated using the extinction coefficient of H_2O_2 (0.071) and the activity was expressed as micromoles of H_2O_2 oxidised per minute.

Results

Thiobarbituric acid reactive substance

Results of ethanol and graded doses of *O. gratissimum* extract on TBARS are presented in Table 1. These results indicate the deleterious effect of free radicals generated by alcohol metabolism. The results were significant ($P<0.01$) at a dose of 80 mg/kg body weight.

Alanine transaminase and aspartate transaminase

Results of the effect of *O. gratissimum* extract on ALT and AST are also presented in Table 1. Observations in the experimental groups (E1–E4) indicate a decreasing trend in enzyme levels as the extract dose increases. Levels at the highest dose (80 mg/kg body weight) showed the most significant results (ALT: $P<0.05$; AST: $P<0.01$).

Reduced glutathione

Results of the effect of ethanol and leaf extract on GSH are shown in Table 2. A significant reduction was seen in the AC group ($P<0.001$). However, although levels were maintained in all the experimental groups (E1–E4), significant differences were noted between the four experimental groups (E1–E3: $P<0.05$; E4: $P<0.01$) and the AC group. No significant difference was noted between the NC group and three out of four experimental groups (E1–E3).

Table 1. Effect of graded doses of *Ocimum gratissimum* on plasma marker parameters (TBARS, ALT and AST) in rats administered alcohol.

	TBARS (mmol/dL) Mean±SE	ALT (U/L) Mean±SE	AST (U/L) Mean±SE
NC	0.19±0.03	30.39±1.43	65.36±3.36
AC	1.30±0.09	49.39±2.01	92.31±2.09
E1	1.01±0.11	47.36±1.39	73.19±3.07
E2	0.95±0.03	40.08±2.17	70.34±1.97[a]
E3	0.23±0.08	39.63±1.05[*]	69.66±1.71[*]
E4	0.17±0.05	33.53±1.91[*]	59.03±2.17[†]

NC: Normal control rats received distilled water.

AC: Alcohol control rats received alcohol (5 g/kg body weight).

E1, E2, E3 and E4: Experimental groups received alcohol (5 g/kg body weight) and methanol extracts (10, 20, 40 and 80 mg/kg body weight, respectively).

[*]$P<0.05$, [†]$P<0.01$.

Table 2. Effect of graded doses of *Ocimum gratissimum* on plasma antioxidants in rats administered alcohol.

	GSH (mg/dL) Mean±SE	Catalase (U/dL) Mean±SE	Vitamin C (mg/dL) Mean±SE
NC	18.50±1.37	71.50±2.98	2.30±0.31
AC	10.67±1.01	42.39±2.94	0.50±0.02
E1	20.67±2.01[*]	49.50±5.34	1.10±0.07[†]
E2	21.39±2.30[*]	50.37±3.67	1.35±0.06[†]
E3	25.63±2.58[*]	76.57±2.57[*]	0.99±0.04[†]
E4	27.30+1.98[†]	85.30±2.67[*]	1.85±0.02[†]

NC: Normal control rats received distilled water.

AC: Alcohol control rats received alcohol (5 g/kg body weight).

E1, E2, E3 and E4: Experimental groups received alcohol (5 g/kg body weight) and methanol extracts (10, 20, 40 and 80 mg/kg body weight, respectively).

[*]$P<0.05$, [†]$P<0.01$.

Catalase

Results for catalase are also presented in Table 2. The leaf extract showed a dose response effect. No significant effect was seen at 10–20 mg/kg body weight, but increased enzyme activity was noted at doses of 40–80 mg/kg body weight ($P<0.05$).

Ascorbic acid

Results for plasma ascorbic acid are also presented in Table 2. A statistically significant difference ($P<0.001$) was seen between the AC group and the NC group. Also, a significant difference was noted between all experimental groups (E1–E4) and the AC group ($P<0.001$).

Discussion

Ethanol metabolism generates free radicals that result in degeneration of hepatic cells, due to alcohol-induced lipid peroxidation.[3,15] This is evident from the level of TBARS in the AC group. Administration of the leaf extract reduced TBARS level in the experimental groups. This demonstrates the preventive and protective effect of the methanol extract of *O. gratissimum*, which occurs in a dose-dependent manner.

The extract also reduced ALT and AST levels in the experimental groups. These enzymes are localised in hepatic cells and leak into the blood after hepatic cell damage. Therefore, high levels of these enzymes in the AC group are indicative of liver cell damage.

It appears that free radicals generated as a result of alcohol metabolism are scavenged either by the compounds present in the plant itself or by a boost to the antioxidant system provided by the extract. Non-enzymatic antioxidants are GSH, ascorbic acid and tocopherol, all of which protect cells from oxidative damage.[11,16]

In the AC group, levels of GSH and ascorbic acid were depleted by more 50%, due to their use in scavenging free radicals. Levels of GSH and ascorbic acid in the NC group and in the experimental groups were protected by participation of *O. gratissimum* in protection against free radicals generated during alcohol metabolism. Furthermore, GSH maintains the levels of ascorbic acid by neutralising free radicals.[17, 18]

It appears that *O. gratissimum* either participates directly in neutralising free radicals, thereby normalising GSH and ascorbic acid levels, or enhances the synthesis of these two antioxidants.

Catalase is an enzymatic antioxidant that helps to neutralise the toxic effect of H_2O_2.[19] Hydrogen peroxide alone does not initiate the chain of events in the lipid peroxidation reaction, but combination with superoxide radicals produces hydroxyl radicals, which are highly reactive and thus initiate the lipid oxidation reactions.

Catalase converts H_2O_2 to water and non-reactive oxygen species, thus it prevents the generation of hydroxyl radicals and protects cells from oxidative damage.

In the present study, catalase activity was very low in the AC group. This could have been due to its active involvement in the detoxification of H_2O_2 generated after alcohol metabolism, and hence its depletion.

In conclusion, the present study demonstrates clearly that *O. gratissimum* can play a significant role in preventing lipid peroxidation and can protect against alcohol-induced hepatotoxicity and liver damage. Furthermore, it enhances the body's antioxidant system by increasing the activity of glutathione, ascorbic acid and catalase. □

The authors are grateful to the University of Botswana for funds to carry out this work, and to Mrs. R. Namposya-Daka for assistance in preparing the manuscript.

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