Analgesic and Hepatoprotective Activity of Methanolic Leaf Extract of Ocimum gratissimum (L.)

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ABSTRACT

The methanolic extract of Ocimum gratissimum (L.) leaves was screened for analgesic and hepatoprotective activity in albino rats, respectively. The use of the hot-plate method to study central analgesic activity of the leaves extract in albino rats indicated that the extract possesses the ability to significantly reduce pain threshold and also increase the response latency period to thermal stimuli in albino rats, similar to the reference drug acetylsalicylic acid. After treatment reaction time of albino rats was significantly increased to 10.92 sec with 40 mg kg⁻¹ of leaves extract, whereas acetylsalicylic acid also increased reaction time to 12.53 sec with 25 mL kg⁻¹. A decline in the reaction time beyond 1.61 sec was observed by the reference drug and leaves extract. Albino rats whose livers were damaged with a hepatotoxin-Carbon tetrachloride (CCL₄) 0.5 mL kg⁻¹ i.p. were used to test for hepatoprotective properties of the plant leaves extract. It reduced significantly (p<0.05) liver enzyme levels for animals treated with CCL₄ (0.5 mL kg⁻¹) and the methanolic plant leaf extract (40 mg kg⁻¹) concurrently compared to animals treated with CCL₄ only. Many histopathological changes in the liver such as marked dilation of the central vein, blood vessel congestion and inflammatory leucocytic infiltrations which were observed in the CCL₄ treated animals were not observed in the CCL₄ + plant extract treated animals. No apparent disruptions of the normal liver structure by histological and enzyme activities assessment were observed. The results show that the methanolic leaf extract is a potent analgesic and antihepatotoxic agent.

Key words: Pain perception, liver enzymes, histology, hepatoprotective, ethnomedicine, bioactivity

INTRODUCTION

The direct use of plants for treatment of ailments is as old as man itself. In the third world countries, medicinal plants are of immense importance in the daily life of the people. Many plants are used as remedies and it is estimated that about 80% of the people in developing countries depend on traditional healing (WHO, 2001). Plants constitute an important source of active natural products that differ widely in terms of their structure and biological properties. Plants have played remarkable roles in the traditional medicine of various countries. The prevention of cancer and cardiovascular diseases has been associated with the ingestion of fresh fruits, vegetables or teas rich
in natural antioxidants (Virgili et al., 2001; Singh et al., 2004). Medicinal plant products have protective effects because of the presence of several components which have distinct mechanisms of action. Some of these components are enzymes and proteins and others are low molecular weight compounds such as vitamins, carotenoids and flavonoids (Zhang and Wang, 2002). Some of these components especially phytochemicals can significantly reduce the risk of cancer due to polyphenol antioxidant and anti-inflammatory effects, antihypertensive (Lee et al., 2001), antihyperglycemic (Jeppesen et al., 2002). Preclinical investigations also suggest that phytochemicals can prevent colorectal cancer and other cancers (Birt et al., 2001; Higdon et al., 2007). Many plants and plant products are recommended for the treatment of liver diseases and most times are claimed to offer significant relief (Arulkumaran et al., 2009). O. gratissimum is one of those plants that have been recommended by traditional medicine practitioners in the South East of Nigeria (Adeyemi et al., 2002).

Ocimum gratissimum (L.) is an erect multi-branched perennial shrub that grows up to a height of two meters with a tap root and many adventitious rootlets. The leaf is rich in essential oils especially thymol, others include flavonoids, saponins, steroids, camphor, estragol, litral, anethol, hydrocynamate and terpenes (Oforkansi et al., 2005). The plant has both culinary and medicinal uses. It is mainly used as a spice to flavor foods and meat. The components of O. gratissimum especially thymol has biological activity such as antiseptic, antitussive, antihelmintic, antispasmodic and antimicrobial. The fresh leaves are used as a laxative, while its infusion serves as a relief for headaches, fever, diarrhea, dysentery, pile and convulsion (Danziel, 1996). In Igbo area of South Eastern Nigeria the leaves extract are applied externally in treatment of conjunctivitis, rheumatic pain, dressing of wounds and lumbago (Iwu, 1993).

Analgesics that act on pain perception within the central nervous system are used mainly to relieve pain that originate in the viscera or arise from severe injuries, burns or neoplasm (Adeyemi et al., 2002).

Carbon tetrachloride (CCl₄) is a hepatotoxin which can have adverse effect on the liver. The affected group of cells may be very small in number or may be of grave and catastrophic proportion as in acute yellow atrophy of the liver where 90% of the parenchyma cells are destroyed. It is known to induce oxidative stress and causes liver injury by formation of free radicals (Manna et al., 2006). The mechanism of Carbon tetrachloride induced hepatic injury is mediated by a reactive metabolite trichloromethyl free radical which is formed by the hemolytic cleavage of carbon tetrachloride:

$$\text{CCl}_4 \rightarrow \text{CCl}_3 + \text{Cl}^-$$

Trichloromethyl peroxy free radical is more reactive than carbon tetrachloride. The toxicity produced by carbon tetrachloride is thought to be due to the reaction of free radicals with lipid and protein. These free radicals cause the peroxidation of the polyenoic lipids of the endoplasmic reticulum and the generation of secondary free radicals derived from these lipids starts a chain reaction (Valko et al., 2007).

The effectiveness of Ocimum gratissimum (L.) as a therapy and in offering any protection against the hepatotoxin depends largely on its ability to either inhibit the activation of carbon tetrachloride to its reactive metabolite carbon trichloride (CCl₃) or its central uptake by the liver free radical generating system (Lee et al., 2004).
The present study is therefore designed to evaluate the analgesic and hepatoprotective effects of the methanolic leaves extract of *O. gratissimum* (L.) in albino rats against claims by ethno-medicine practitioners that the plant leaf has both analgesic and hepatoprotective properties.

**MATERIALS AND METHODS**

**Plant materials:** Fresh leaves of *O. gratissimum* (L.) were collected from plants growing in the campus of Abia State University, Uturu, Nigeria as ornamental plants on the 24th of February 2011. The plant was authenticated at the Department of Plant Science and Biotechnology, Abia State University, Uturu, Nigeria. Voucher specimen of the plant was deposited at the departments' herbarium.

**Preparation of methanol extract:** Samples of the fresh leaves were first washed with distilled water to remove dirt and contaminants. They were oven dried at a temperature of 27°C for three days. The dried sample was then pulverized into fine powder. About 600 g of the milled sample was extracted in methanol 100 mL using the Soxhlet apparatus. The volume of the extract collected was 60.5 mL. Standardization of the extract gave 9.16 mg mL⁻¹.

**Experimental procedure:** Approval for animal studies was obtained from the College of Health Sciences, Animal Ethics Committee, Abia State University, Uturu. Inbred male albino rats weighing between 150 to 200 g, aged six weeks were used for the investigation. The animals were housed in standard cages and maintained on standard rat pellets and water ad libitum. Twenty four hours before the experiment, food was withheld but the animals had free access to water. The animals were randomly divided into five groups of five animals each. Groups one to three received 100, 200, 300 mg kg⁻¹ of the extract, respectively. Group four received 25 mL kg⁻¹ of acetylsalicylic acid, while group five a placebo of normal saline (0.5 mL) which is the vehicle for the extract as control by oral administration. Two end points were used in the investigation; Pre-treatment Reaction Time (PRT) and After Treatment Reaction Time (ATRT).

**Analgesic studies:** Analgesic effect was investigated by inducing pains in the animals through thermal means as recommended by International Association for the study of pain (Annegowda *et al.*, 2010) and the guidelines on ethical standards for the investigation of pain in animals (Bhandare *et al.*, 2010).

**Hepatoprotective studies:** Albino rats (males) weighing between 145 and 160 g and aged six weeks were used to test for hepatoprotective effect. The animals were divided randomly into three groups consisting of five animals each. Group 1 served as control which received a placebo of normal saline (0.5 mL) which is the vehicle for the extract for 7 days. Group 2 received CCl₄ 0.5 mL kg⁻¹ i.p. for 7 days while group 3 received CCl₄ 0.5 mL kg⁻¹ i.p. and the plant extract (40 mg kg⁻¹ orally) concurrently for 7 days. After 7 days of treatment, the rats were kept overnight fasting and killed by cervical dislocation. Blood samples were collected by direct cardiac puncture under ether anesthesia and the serum was used for the assay of the marker enzymes. Serum Aspartate Aminotransferase (AST), Serum Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) were determined using Randox diagnostic kit. Determination was based on the principle described by Reitman and Frankel (1957).
Histopathological studies: Histological studies on the liver were done according to procedures described by Dacie and Lewis (1991) and Disbrey and Rack (1970).

Statistical analysis: Values were represented as Mean±SD. Data obtained were subjected to one way Analysis of Variance (ANOVA) and group means were compared using Duncan's multiple range tests. p-values (p<0.05) were considered significant.

RESULTS AND DISCUSSION
Histopathological examination: Microscopic examination of the control group liver, Fig. 1a showed a normal morphology with the central vein in the centre of the lobule. The liver cells are radially arranged to form sheets. The venous sinusoids converge upon the central vein. The liver cells possess distinct cell outline.

Histopathological examination of CCl₄-treated rat liver, Fig. 1b revealed centrilobular fatty degeneration and swelling, hepatic damage, damaged sinusoidal architecture with broad patches of hepatic cellular necrosis and fibrosis.

In liver histology of animals treated with CCl₄-plant extract, Fig. 2a and b show normalization of hepatic cells, central vein and portal triad. Liver histology improved as compared to CCl₄ treated animals with few necrotic patches and very little fatty change. The lamina of the hepatic cells is well-defined. Concurrent administration of CCl₄ and O. gratissimum (L.) leaf extract preserved the histological structure of liver though there was mild congestion and regeneration of liver tissue.

Table 1 shows the result on the analgesic activities of methanolic leaf extract of O. gratissimum (L.). With the hot plate method, the rats were affected by heat while the extract reduced the

Fig. 1 (a-b): (a) Normal control liver and (b) Liver of animals treated with CCl₄. Photomicrophotographs of a cross-section of liver of rats. MAGx40

Fig. 2 (a-b): Photomicrophotographs of a cross-section of liver of rats administered CCl₄-plant extract. MAGx40
perception of pain (group 1) mean PRTT 3.4±0.15 sec while mean ATRT was 10.92±0.2 sec compared to (group 5-positive control) PRTT 3.6±0.41 sec and ATRT 7.15±0.378 sec. This shows it took a significantly (p<0.05) longer time for the animals to feel the heat when the O. gratissimum (L) leaf extract was administered. Table 1 also shows the effect of acetylsalicylic acid on pain perception in the animals (group 4 animals). PRTT 3.5±0.32 sec and ATRT 12.53±1.27 sec. This result shows that the drug (acetylsalicylic acid) had analgesic effect on the animals compared to the plant extract (group 1-3).

A comparison of mean ATRT for the administered extract (Table 1) 10.92±0.21, 11.20±0.11 and 11.56±0.15 sec and mean ATRT for the administered drug (group 4) 12.53±1.27 sec shows that the acetylsalicylic acid had a non-significantly (p<0.05) greater analgesic effect compared to leaf extract of O. gratissimum (L) which is a decline of about 1.61 sec. Our findings seem to be in agreement with those of Chandrashekar et al. (2004) who reported that an analgesic is considered positive when the animal fails to respond to painful stimulus for a period corresponding to the PRTT plus 4 sec. Adeyemi et al. (2002) and Choi and Hwang (2004) also reported similar values for analgesic activity of Persea Americana and Foeniculum vulgare, respectively. In this study the results of 10.92±0.21 sec as ATRT and 3.4±0.15 sec as PRTT (Table 1) seem to agree with this theory.

Table 2 shows that carbon tetrachloride (CCL₄) caused liver damage as is evident in the significantly (p<0.05) high concentration of hepatic enzymes in the CCl₄ treated rats (group 2 animals), 48.6±3.84, 54.4±2.62 and 55.4±0.27 U L⁻¹ for serum aspartate aminotransferase; serum alanine aminotransferase and serum alkaline phosphatase, respectively, as compared to the control (group 1), 25.8±1.26, 27.0±1.14 and 26.2±1.32 U L⁻¹ for AST, ALT and ALP, respectively. Measurement of enzyme activities in tissues and body fluids play significant roles in disease investigation, diagnosis and detection of tissue cellular damage (Chang et al., 2004). It is quite possible that the increase in activity of these enzymes may be due to the hepatotoxic effect of carbon tetrachloride on the liver (Weber et al., 2003). Adesokan and Akanji (2003) and Jensen and Freeese (2009) also reported the significant increase of serum aspartate aminotransferase and serum alanine aminotransferase levels by hepatotoxins.

For the group 3 animals treated with 0.5 mL kg⁻¹ of CCL₄ and 100 mg kg⁻¹ of the extract of O. gratissimum (L) concurrently, the enzyme concentrations were significantly (p<0.05) reduced 28.4±2.35, 31.2±2.86 and 30.2±0.01 U L⁻¹ for AST, ALT and ALP, respectively (Table 2). The

<table>
<thead>
<tr>
<th>Drug and dose (n = 5)</th>
<th>PRTT (sec)</th>
<th>ATRT (sec)</th>
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<tr>
<td>Group 1 100 mg kg⁻¹ of extract</td>
<td>3.4±0.15</td>
<td>10.92±0.21</td>
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<tr>
<td>Group 2 200 mg kg⁻¹ of extract</td>
<td>3.5±0.31</td>
<td>11.20±0.11</td>
</tr>
<tr>
<td>Group 3 200 mg kg⁻¹ of extract</td>
<td>3.2±0.25</td>
<td>11.56±0.15</td>
</tr>
<tr>
<td>Group 4 25 mL kg⁻¹ of drug</td>
<td>3.6±0.32</td>
<td>12.53±1.27</td>
</tr>
<tr>
<td>Group 5 0.5 mL saline</td>
<td>3.6±0.41</td>
<td>7.15±0.36</td>
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</tbody>
</table>

*Values are Means±SD of triplicate determinations. PRTT: Pretreatment reaction time. ATRT: After treatment reaction time

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Group 1 (Control)</th>
<th>Group 2</th>
<th>Group 3</th>
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<tr>
<td>AST</td>
<td>25.8±1.26</td>
<td>48.6±3.84</td>
<td>28.4±2.35</td>
</tr>
<tr>
<td>ALT</td>
<td>27.0±1.14</td>
<td>54.4±2.62</td>
<td>31.2±2.86</td>
</tr>
<tr>
<td>ALP</td>
<td>26.2±1.32</td>
<td>55.4±0.27</td>
<td>30.2±0.01</td>
</tr>
</tbody>
</table>

*Values are Means±SD of triplicate determinations (n = 5). AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. ALP: Alkaline phosphatase
reduction in enzyme concentration for the animals co-administered CCL₄ and the leaf extract (group 3) is significant (p<0.05) when compared to enzyme concentrations of animals administered CCL₄ only (group 2 animals): 48.6±3.84, 54.4±2.62 and 55.4±0.27 U L⁻¹ for AST, ALT and ALP, respectively. The significant (p<0.05) reduction in enzyme concentrations suggests that methanolic leaves extract of *O. gratissimum* (L.) has hepatoprotective effect and clearly showed that this is a preventive action on damaged liver by *O. gratissimum* leaf extract.

The co-administration of the leaf extract and carbon tetrachloride revealed remarkable inhibition of CCL₄ hepatotoxicity. The significantly (p<0.05) low concentrations of the liver enzymes in group 3 animals compared to those of group 2 showed the ability of the plant extract to inhibit the NADPH₂-ADP-Fe²⁺ system which is responsible for the activation of CCL₄ as reported by Chandra Shekar and Prasanna (2010). Among the various methods involved in the hepatotoxic effect of carbon tetrachloride, one is oxidative damage through free radical generations (Ali et al., 2009; Dhanasekaran and Ganapathy, 2011) and antioxidant property is claimed to be one of the mechanisms of hepato-protective effect of indigenous drugs (Chandra Shekar and Prasanna, 2010). Some investigators have also made similar reports on medicinal plants, such as leaves of *Melia azedarach* and seeds of *Piper longum* (Samudram et al., 2008), *Chamomile recutita* (Gupta et al., 2006) and *Terminalia arjuna* (Venkatesh et al., 2007) and Coptidis rhizome (Ye et al., 2009).

Reports on effect of long term consumption of *O. gratissimum* in male rats shows the animals had increased weight, reduction in serum protein, cholesterol, lipid peroxidation and haemoglobin. Superoxide dismutase was significantly increased but changes in glutathione-s-transferase, ALT, AST and ALP were not significant (Iweala and Obiedo, 2010). Efrain et al. (2003) reported that at high concentrations the *O. gratissimum* leaf extract could be toxic. The essential oil in *O. gratissimum* has been associated with its toxicity (Orafidiya et al., 2004).

**CONCLUSION**

The results from this study showed the *O. gratissimum* plant leaf extract increased pain perception time comparable to the drug. It also decreased hepatic enzymes when administered concurrently with carbon tetrachloride in animals as histopathological observations showed remarkable reverting changes in liver sections of the *O. gratissimum* treated group than in the untreated group. Enzyme analyses were also in good agreement with biochemical changes. In conclusion the present study demonstrates that *O. gratissimum* methanolic leaf extract has analgesic properties and hepatoprotective effect against CCL₄ induced hepatotoxicity which is preventive. The *O. gratissimum* leaves may be used to achieve these objectives as practiced by ethno-medicine practitioners. *O. gratissimum* should have potential for developing drug for liver disorders.

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