The effects of *Cymbopogon citratus* (Lemon grass) on the antioxidant profiles wistar albino rats

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INTRODUCTION

*Cymbopogon citratus* commonly called lemon grass is an aromatic, perennial grass belonging to the family grimeae (Ebomoyi, 1986). It is a tropical plant, grown as an ornamental in many temperate areas with maximum a height of about 1.8m and its leaves 1.9cm wide covered with a whitish bloom (Gbile, 1986). In certain medications, it is used for mental illness. It is an antifungal, anti-toxicant and deodorizing agent. In combination with other herbs, it has large use as cure for Malaria (Gbile, 1986).

One of the main constituents of the many different species of lemongrass (genus *Cymbopogon*) is citral (3,7-dimethyl-2,6-octadien-1-al) (Balbaa and Johnson, 1955; Banthorpe et al., 1976). Lemongrass oil has been found to contain up to 75-85% citral (Nhu-Trang et al., 2006). Lemongrass also contains z-citral, borneol, estragole, methyleugenol, geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate), geranial (some species higher in this compound than citral), beta-myrcene (MYR, 7-methyl-3-methylene-1,6 octadiene), limonene, piperitone, citronellal, carene-2, alpha-terpineole, pinene, farnesol, proximadiol, and (+)-cymbodiaceatal (Hegnauer, 1955). The volatile oil from the roots contains 56.67% longifolene-(V4) and 20.03% selina-6-en-4-ol (Li et al, 2005). In particular, a study of *Cymbopogon martini* isolated fatty acids, common sterols, and 16-hydroxypentacos-14(2)-enoic acid (Siddiqi and Misra, 2000).

An antioxidant is a molecule, which terminate chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thios, ascorbic acid, or polyphenols (Sies, 1997). Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness (Baillie et al., 2009).

The reactive oxygen species produced in cells include
hydrogen peroxide (H$_2$O$_2$), hypochlorous acid (HClO), and free radicals such as the hydroxyl radical (·OH) and the superoxide anion (O$_2^-$) (Valko et al., 2007). The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins (Sies et al., 1997).

The purpose of this study, therefore, is to carry out the biochemical studies on the effects of *cymbopogon citratus* (lemon grass) on wistar albino rats.

**MATERIALS AND METHODS**

*Cymbopogon citratus* was harvested and collected freshly from a native farms and authenticated in Environmental Biology Laboratory, Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo.

**Preparation of plant extract**

The fresh plant was washed, chopped into pieces and air-dried at room temperature. The dried plant part was milled into powder and weighed. The Plant powder was divided into two groups. One portion was soaked in 90% absolute ethanol to obtain the ethanolic extract and the other group in distilled water to obtain the aqueous form separately in a container for 72 hours with intermittent shaking. Then, it was filtered through a muslin clothe and later Whatman No. 1 filter paper.

The resulting filtrate was evaporated under reduced pressure using a rotary evaporator and then after freeze dried to get powder form of both ethanolic and aqueous extracts. The yield was stored in a refrigerator (4°C) till when needed (Onoagbe and Esekheigbe, 1999).

**Experimental animal**

Male albino rats (Wistar strain) weighing between 109-170g, purchased from the central animal house of University of Ibadan were used for the study.

**Acclimatization**

15 days prior to dosing.

**Identification of animals**

By cage number.

**Diet**

Pelleted feed

**Water**

Potable drinking water

**Housing and Environment**

4 animals each in a group

**Determination of the weight of animals**

The weights of the animals were weighed using an electronic weighing balance every 7 days to verify and quantitate the change in weight over the period of administration.

**Animal ethics**

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (USA). The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments.

**Experimental design**

To carry out Biochemical studies on the effects of both ethanolic and aqueous extracts, twelve male Albino rats were randomly, equally divided and assigned to either control or experimental groups. The control group received 2ml distilled water while the experimental rats group received oral doses of 200mg/kg for both the ethanolic and aqueous extracts of *Cymbopogon citratus* dissolved in 2ml distilled water through a stainless steel intra-gastric intubation and administered for 30 days. Here, antioxidants profiles and Histopathology photomicrograph were determined and recorded.

**Chemicals and reagents preparation**

All chemicals were of an analytical grade and are supplied from sigma chemical co. USA. Distilled water was used in all biochemical assays.

2-Acetylaminofluorene (2-AAF), Luteolin (3’,4’,5,7-tetrahydroxyflavone), dimethylsulphoxide (DMSO), 5’5’-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione
Table 1. Effects of oral administration of ethanolic and aqueous extracts of *Cymbopogon citratus* on the weight of normal rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ethanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>115 ± 1.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130 ± 1.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>135 ± 1.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>129 ± 1.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150 ± 1.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>149 ± 1.70&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM of four independent experiments. Means in the same column not sharing the same letter(s) are significantly different (p < 0.05)

Table 2. Effects of oral administration of ethanolic extracts of *Cymbopogon citratus* on the weight of organs in normal rats

<table>
<thead>
<tr>
<th>Group (mg/kg)</th>
<th>Liver (g)</th>
<th>Kidney (g)</th>
<th>Heart (g)</th>
<th>Pancrease (g)</th>
<th>Spleen(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.98 ± 0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40 ± 0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.79 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanolic extract (200mg/kg)</td>
<td>3.71 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.90 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aqueous extract (200mg/kg)</td>
<td>5.12 ± 0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.45 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.70 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM of four independent experiments. Means in the same column not sharing the same letter(s) are significantly different (p < 0.05)

(GSH), glucose-6-phosphatase, hydrogen peroxide, Sodium chloride (NaCl), ethylenediamine tetraacetic acid (EDTA), Sodium hydroxide (NaOH), Copper sulphate (CuSO<sub>4</sub>), magnesium chloride (MgCl<sub>2</sub>), glacial acetic acid, trichloroacetic acid (TCA), Sodium acetate, Ammonium molybdate, Ferrous sulphate, Sodium-potassium tartarate, Bovine Serum Albumin (BSA), Potassium iodide (KI), Potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>), thiobarbituric acid (TBA), Alanine aminotransferase (ALT) randox kit, Aspartate aminotransferase (AST) randox kit, γ-glutamyltransferase (γ-GT) randox kit, Butylated hydroxyanisole (BHA).

Biochemistry assays

Superoxide dismutase (SOD) (Misra and Fridovich, 1972), Catalase (Sinha, 1971), reduced glutathione (GSH) (Beutler *et al.*, 1963) and Vitamin C (AOAC, 1995).

Organs preparation

Rat organs (liver, kidney, heart, pancreas and spleen) were used to determine the antioxidant profiles.

At the end of the experiment, rats were fasted for 12 to 14 h. Rats were sacrificed cardiac puncture from the rats at fasting state after being anesthetized with chloroform. The organs were collected in plain tubes, homogenised at room temperature and centrifuged at 3500 rpm for 15 min at room temperature for separation. The clear, non-haemolysed supernatant was separated using clean dry Pasteur pipette and stored at -20°C.

Histopathology

Small pieces of tissues were collected in 10% formaldehyde solution for histopathological study. The pieces of the liver was soaked in formalin for 6 hrs, embedded in paraffin wax and the sections were made about 4-6µm in thickness. They were stained with hematoxylin and eosin and photographed (Arthur and John, 1978).

Statistical analysis

The experimental results were expressed as the mean ± S.E.M. Statistical significance of difference in parameters amongst groups was determined by One way ANOVA followed by Duncan’s multiple range test. P<0.05 was considered to be significant.

RESULTS AND DISCUSSION

In the liver, a significant (p>0.05) increase was observed in the level of SOD, catalase, GSH and vitamin C level in both ethanolic and aqueous extracts of *C. citratus* as compared with the control. In the kidney, a significant (p>0.05) increase was observed in the level of SOD, catalase, GSH and vitamin C level in both ethanolic and aqueous extracts of *C. citratus* as compared with the control. In the pancreas, a significant (p>0.05) increase was observed in the level of SOD, catalase, and GSH level in both ethanolic and aqueous extracts of *C. citratus* as compared with the control. (Tables 1-6)
Table 3. Effects of oral administration of ethanolic and aqueous extracts of *Cymbopogon citratus* on Antioxidant profiles in the liver in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (Unit/g protein)</th>
<th>Catalase (ml/min/g protein)</th>
<th>GSH (mg/g protein)</th>
<th>VitaminC (mmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.76 ± 1.60</td>
<td>0.018 ± 0.003</td>
<td>1.85 ± 0.37</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>7.42 ± 0.21</td>
<td>0.025 ± 0.001</td>
<td>2.15 ± 0.46</td>
<td>0.10 ± 0.0014</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>6.88 ± 0.55</td>
<td>0.021 ± 0.001</td>
<td>1.92 ± 0.00</td>
<td>0.12 ± 0.06</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM of four independent experiments. Means in the same column not sharing the same letter(s) are significantly different (p < 0.05)

Table 4. Effects of oral administration of ethanolic and aqueous extracts of *Cymbopogon citratus* on Antioxidant profiles in the kidney in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (Unit/g protein)</th>
<th>Catalase (ml/min/g protein)</th>
<th>GSH (mg/g protein)</th>
<th>VitaminC (mmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.07 ± 1.35</td>
<td>0.028 ± 0.002</td>
<td>1.20 ± 0.26</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>16.46 ± 2.94</td>
<td>0.035 ± 0.001</td>
<td>1.34 ± 0.41</td>
<td>0.11 ± 0.06</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>14.63 ± 1.46</td>
<td>0.034 ± 0.003</td>
<td>1.75 ± 0.30</td>
<td>0.91 ± 0.03</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM of four independent experiments. Means in the same column not sharing the same letter(s) are significantly different (p < 0.05)

Table 5. Effects of oral administration of ethanolic and aqueous extracts of *Cymbopogon citratus* on Antioxidant profiles in the Pancrease in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (Unit/g protein)</th>
<th>Catalase (ml/min/g protein)</th>
<th>GSH (mg/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.70 ± 0.61</td>
<td>0.024 ± 0.006</td>
<td>1.30 ± 0.02</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>16.76 ± 0.35</td>
<td>0.028 ± 0.004</td>
<td>1.37 ± 0.24</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>15.60 ± 1.05</td>
<td>0.026 ± 0.008</td>
<td>1.34 ± 0.05</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM of four independent experiments. Means in the same column not sharing the same letter(s) are significantly different (p < 0.05)

SOD- Superoxide dismutase, GSH – Reduced glutathione.

Table 6. Effects of oral administration of ethanolic and aqueous extracts of *Cymbopogon citratus* on Antioxidant profiles in the Pancrease in normal rats

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (Unit/g protein)</th>
<th>Catalase (ml/min/g protein)</th>
<th>GSH (mg/g protein)</th>
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<tr>
<td>Aqueous extract</td>
<td>15.60 ± 1.05</td>
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</tr>
</tbody>
</table>

Values are expressed as means ± SEM of four independent experiments. Means in the same column not sharing the same letter(s) are significantly different (p < 0.05)
Figure 1. Histopathology photomicrograph of Liver tissue of albino rats in subchronic treatment with ethanolic and aqueous extracts of *Cymbopogon citratus* (H and E stain) mag. x200.

Figure 2. Histopathology photomicrograph of Heart tissue of albino rats in subchronic treatment with ethanolic and aqueous extracts of *Cymbopogon citratus* (H and E stain) mag. x200.

Figure 3. Histopathology photomicrograph of Kidney tissue of albino rats in subchronic treatment with ethanolic and aqueous extracts of *Cymbopogon citratus* (H and E stain) mag. x200.
From Figure 1 above, the control group shows no visible lesions in the liver tissue of the rats. The 200mg/kg ethanolic extract treated group, liver tissue of rats showed a generalized congestion of the blood channels while the aqueous extract treated group showed a moderate congestion of the central veins.

From Figure 2, in the control, the heart tissue of rats showed no visible lesions. While the ethanolic extract treated group showed a generalised congestion of the blood channels and aqueous extract treated group showed a moderate congestion of the central veins in the heart.

From Figure 3 above, Kidney tissues of control group showed no visible lesions. While the 200mg/kg ethanolic extract treated group showed a severe congestion of the entire parenchyma aqueous extract of C. citratus treated rats showing no visible lesion.

Figure 4 above the spleen tissue of control rats showed no visible lesion. The 200mg/kg ethanolic extract treated group showed a severe congestion of the entire parenchyma aqueous extract of C. citratus treated rats showing no visible lesion.

Thus, the histopathological results showed that no degenerative conditions in the liver, Heart or spleen and no necrotic changes in the tubular epithelia of the kidney with cellular infiltration was observed in the all the treated groups when compared against control.

DISCUSSION

Many indigenous medicinal plants have been reported by various authors to have hypoglycemic effects (Young and Maciejewski, 1997). Some of these hypoglycemic medicinal plants have been shown to significantly reduce blood glucose concentration in normal and diabetic animals. These plants, for example C. citratus, tend to participate in the tight regulation of blood glucose levels as a part of metabolic homeostasis.

In this study, table 3, 4, 5 and 6 showed the effects of both the ethanolic and aqueous extracts of C. citratus on the oxidative status of normal rats were determined in the liver, kidney, and pancreas for 30 days by measuring activities of superoxide dismutase (SOD), catalase, Glutathione reductase (GSH) and Vitamin C. This investigation is part of a biochemical evaluation of these medicinal plants, in order to ascertain their efficacy and safety in managing diabetes mellitus.

Mahdi et al. (2003) reported that three of the four hypoglycemic plants they studied significantly increased superoxide dismutase activity compared to diabetic control, implying that the plants improved the oxidative status of the diabetic subjects; the fourth hypoglycemic plant did not have any effect on SOD activity. The results obtained in our study indicate that oxidative status of normal rats was normal. The observation that the tests animals had serum SOD activities that were comparable to control implies that the medicinal plants did not negatively alter their oxidative status. Apart from kidney SOD treated rats that were increased; most of the tissue SOD activities recorded were similar to control. Panda and Kar (1998) reported significantly increased activity of two antioxidant enzymes in liver i.e. SOD and catalase following treatment with aqueous extract of Ocimum sanctum. The increases observed in this study correlates well with their observation, implying that the medicinal plants used in this study enhanced the oxidative status of some tissues.

Adewole and Caxton-Martins (2006), reported that the hypoglycemic plant they studied, Annona muricata Linn, significantly (p<0.05) enhanced the activities of the antioxidant enzymes catalase, glutathione peroxidase and SOD compared to diabetic control. The increases observed in this study agree with their findings,
suggesting again the all three medicinal plants, at some point, enhanced the oxidative status of test animals. Chronic oxidative stress due to hyperglycemia may play an important role in progressive β-cell dysfunction (Tiwari and Rao, 2002; Robertson, 2004), since pancreatic islets have low expression of antioxidant enzymes (Lenzen et al., 1996; Tiedge et al., 1997), the significant increase in pancreas catalase activities seen in this study is particularly advantageous as it may enhance the pancreas’ ability to combat destructive oxidants and improve pancreatic function. Indeed studies have shown that antioxidants can ameliorate β – cell dysfunction (Matsuoka et al., 1997; Tanaka et al., 1999).

In Figure 1, 2, 3, and 4, the histopathological results showed that no degenerative conditions in the liver, Heart or spleen and no necrotic changes in the tubular epithelia of the kidney with cellular infiltration was observed in all the treated groups when compared against control. This effect agree with the theory of target organ toxicity (Heywood, 1981) since the kidney is the organ of excretion (Parke, 1982). Observations of liver histopathology, when subjected to comparison with the histochemical observations of liver, were in complete accordant with each other. There was moderate to normal glycogen localization in almost all hepatocytes following the 30 days administration of the various doses of both the ethanolic and aqueous extracts of *Cymbopogon citratus*.

Meanwhile, the other study by Salawu et al. (2009) using *Crossopteryx febrifuga* saw inflammatory changes histologically in the liver by infiltration of lymphocytes at portal and central of rat treated with at dose level 500 and 1000 mg/kg and this shows that, the extract exerted deleterious effects on the liver. The liver is capable of regenerating damaged tissue, hence the liver function may not be impaired early following an insult from a toxicant (Salawu et al., 2009). Apart from that, the acute toxicity study conducted on *C. fistula* pod extract and histological examination of the organs of rat treated with extract at a dose of 1000 mg/kg revealed that there was no potential toxicity or damage to the cell structure of liver, kidney and testes. Also there was no necrosis, inflammatory reaction, fibrosis or local fatty degeneration observed in liver and the arrangement of cell structure almost similar to the organs of rats in control groups (Akanmu et al., 2004). The histological features liver from this study is displayed in Figure 1. The morphology of liver cell in both control and treated groups are normal and no structural damages were observed.

The kidney micrograph displayed in Figure 3 shows that no adverse effects were observed in both groups and the glomeruli and capsules appeared normal and the Bowman’s space are also marked clearly. In contrast, the study conducted by Alade et al. (2009) revealed the histology of kidney observed with focal proximal tubular epithelial necrosis, meanwhile there was variation in the lung between the controls and treated with rat *B. monandra* leaf extract at dose 4 g/kg.

Furthermore, in this study the microscopic inspection of the heart (Figure 2) of the rats treated with extracts did not indicate any changes in the organs compared to control rats. Meanwhile, histology of the spleen (Figure 4) of rats in both control and treated groups are well labelled with white pulp and red pulp.

**CONCLUSION**

In conclusion, *Cymbopogon citratus* are recommended cardiac glycoside and the cardiac glycosides serves as defence mechanisms against cardiovascular disease and digestive problems. Thus, *Cymbopogon citratus* (Lemon grass) whole plant materials are recommended to be taken because it has many beneficial effects in human health (Ozcan et al., 2009).

**REFERENCES**


