Aqueous and ethanolic leaf extracts of *Ocimum basilicum* (sweet basil) protect against sodium arsenite-induced hepatotoxicity in Wistar rats

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**Summary:** We evaluated the effects of aqueous and ethanolic leaf extracts of *Ocimum basilicum* (sweet basil) on sodium arsenite-induced hepatotoxicity in Wistar rats. We observed that treatment of the animals with the extracts before or just after sodium arsenite administration significantly (p < 0.05) reduced mean liver and serum γ-glutamyl transferase (γGT), and serum alkaline phosphatase (ALP) activities when compared with the group administered the toxin alone. In addition, treatments of the animals with aqueous or ethanolic extract of *O. basilicum* before the administration of sodium arsenite resulted in the attenuation of the sodium arsenite-induced aspartate and alanine aminotransferase activities: ALT (from 282.6 % to 167.7 % and 157.8 %), AST (from 325.1 % to 173.5 % and 164.2 %) for the group administered sodium arsenite alone, the aqueous extracts plus sodium arsenite, and ethanolic extracts plus sodium arsenite respectively, expressed as percentage of the negative control. These findings support the presence of hepatoprotective activity in the *O.basilicum* extracts.

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**Keywords:** *Ocimum basilicum*, hepatotoxicity, sodium arsenite, γ-glutamyl transferase, environmental contaminant

**Abbreviations:** CDNB (1-chloro-2, 4-dinitrobenzene), γGT (γ-glutamyl transferase), GST (Glutathione-S-Transferase),

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**INTRODUCTION**

The role of environmental factors in the aetiology of most genetic diseases cannot be over emphasized. Exposure to environmental contaminants is inadvertent and difficult to control. On the other hand, dietary components/additives have been implicated as protective agents in the control of cancer (Wattenberg 1985, Popkin 2007). Several vegetables and spices are now known to exhibit medicinal, antioxidant and anticarcinogenic properties and effects (Aruna and Sivaramakrishnan 1992, Myagmar and Aniya 2000, Bajpai et al. 2005).

*Ocimum* group of species have been shown to possess a wide range of chemopreventive and medicinal activities (Godhwani et al. 1987, Banerjee et al. 1996, Karthikeyan et al. 1999, Rastogi et al. 2007). In addition, extracts of the leaves displayed powerful antioxidant activity in various assay models (Jayasinghe et al. 2003, Gulcin et al. 2007). *O. basilicum* had been found to contain linalool, eugenol, methyl chavicol, methyl cinnamate, ferulate, methyl eugenol, triterpenoids and steroidal glycoside known to exhibit antioxidant activities (Zheljazkov et al., 2008, Siddiqui et al., 2007a, Siddiqui et al., 2007b). It is therefore possible that the extracts may serve as a remedy by blocking or intercepting the activity of environmentally acquired toxins such as mycotoxins, insecticides and pesticides.

Sodium arsenite is widely used as a component of herbicides, fungicides, insecticides, and algaecides, and in the manufacture of arsenical soap (Griffon et al. 1961, Da Costa, 1972, Shariatpanahi et al. 1981, Chen et al. 2006). Drinking water with a high inorganic arsenic concentration is the main source of arsenic exposure for the world population (Chiou et al. 1995). Studies have proved sodium arsenite to be highly toxic (Biswas et al. 2007, Yousef et al. 2008,
El-Demerdash et al. 2009). Previous studies in our laboratory have shown that it is hepatotoxic and clastogenic in experimental animals (Odunola et al. 2008, Gbadegesin, et al. 2009). The principal mechanism of arsenic intoxication is disruption of thiol proteins (Chouchane and Snow 2001). Sodium arsenite has also been shown to decrease glutathione levels and increase lipid peroxidation in liver, kidney and heart (Lee and Ho, 1994, Ramos et al. 1995).

The objective of this study is to evaluate the effects of aqueous and ethanolic leaf extracts of *O. basilicum* on sodium arsenite induced hepatotoxicity in the Wistar rats. The activities of the enzymes aspartate and alanine aminotransferases, alkaline phosphatase, γ-glutamyl transferase and glutathione-S-transferase were monitored in the treated and control animals.

**MATERIALS AND METHODS**

**Chemicals, Reagents and Test Substances:** All reagents and solvents were of analytical grade. Sodium arsenite (Na₂AsO₂) was from Sigma Chemical Co., St Louis, MO. It was dissolved in distilled water and administered at 2.5 mg/kg body weight as detailed below.

**Plant materials and extraction procedures:** Fresh *O. basilicum* leaves were collected from the University of Ibadan, Ibadan botanical garden and authenticated at the Department of Botany herbarium. The leaves were dried at room temperatures on bench surface at the Department of Biochemistry. The dried leaves were ground into a fine powder in a mill. Aqueous and ethanol extraction methods used were as reported by Gulcin (2005). The solvent were removed using rotary evaporator. The extracts were then suspended in convenient volume of distilled water and administered as shown below.

**Experimental animals and treatments:** Thirty-five male Albino Wistar rats weighing between (130-160) g and of ages between (12 – 15) weeks were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, University of Ibadan, Nigeria. They were housed in steel metal cages in the Animal House, Department of Biochemistry. The rats were fed pellets (from Ladokun Livestock Feeds Limited, Ibadan, Nigeria) and given water *ad libitum*. The animals were randomly assigned into seven groups (A-G) of five rats each and treated as below:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments</th>
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<tbody>
<tr>
<td>A</td>
<td>Distilled water only by oral intubations. This is the negative control group</td>
</tr>
<tr>
<td>B</td>
<td>Sodium arsenite via i.p route at 2.5 mg/kg body weight (1/10 of its LD₅₀). This is the positive control group</td>
</tr>
<tr>
<td>C</td>
<td>Aqueous extract of <em>O. basilicum</em> leaves only p.o. at 400 mg /kg body weight for 14 days: The extracts dose of 400mg/kg body weight was found to be the most effective in the dose range of (100 – 400) mg/kg body weight by Manikandan et al. (2007)</td>
</tr>
<tr>
<td>D</td>
<td>Ethanolic extract of <em>O. basilicum</em> leaves only p.o at 400mg/kg body weight for 14 days</td>
</tr>
<tr>
<td>E</td>
<td>Pre-treatment with aqueous extract of <em>O. basilicum</em> leaves p.o. at 400mg/kg body weight for 14 days + sodium arsenite for the next 14th day at 2.5 mg/kg body weight (i.p)</td>
</tr>
<tr>
<td>F</td>
<td>Pre-treatment with ethanolic extract of <em>O. basilicum</em> leaves p.o for 14 days + sodium arsenite for the next 14th day at 2.5 mg/kg body weight (i.p)</td>
</tr>
<tr>
<td>G</td>
<td>Sodium arsenite at 2.5 mg/kg body weight (i.p) + ethanolic extract of <em>O. basilicum</em> leaves (p.o) at 400mg/kg body weight for 14 days (both arsenite and extract given daily for 14 days)</td>
</tr>
</tbody>
</table>

The animals were allowed to acclimatise for seven days before the commencement of the experiment with 12 hours light/ dark cycle and temperature of 29± 2 °C. These conditions were maintained throughout the duration of the experiment.

**Enzyme Assay**

Gamma-glutamyl transferase (γGT) activity was assayed in the serum and liver homogenates by using the reconstituted γGT diagnostic reagent following the method of Szasz (1969). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed according to Reitman and Frankel (1957). This method involves the reaction of pyruvate, the product of transamination reaction catalysed by ALT or AST, with 2, 4- dinitrophenyl hydrazine to produce intensely coloured hydrazone read at 546 nm using spectronic-20 spectrophotometer. Alkaline phosphatase (ALP) assay was based on the method of Williamson (1972). This is based on spectrophotometric (405 nm) determination of concentration of p-nitrophenol formed by the dephosphorylation of p-nitrophenyl phosphate (PNPP) catalysed by ALP. Hepatic GST activities were determined according to Habig et al. (1974). The cytosolic GST activities were determined using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.
The reaction was followed by measuring the absorbance at 340 nm at 1 minute intervals for 5 minutes. The specific activities were expressed as nmole of CDNB-GSH conjugate formed per mg of protein. Protein determination of each sample was according to Lowry et al. (1951) using BSA as standard.

Statistical Analysis
The data were analysed by One-way Analysis of Variance (ANOVA) followed by Least Significant Difference (LSD). P values less than 0.05 were considered statistically significant. All the results are expressed as mean ± standard deviation.

RESULTS

Leaf extracts of *Ocimum basilicum* suppressed sodium arsenite-induced γ-glutamyl transferase (γGT) and alkaline phosphatase (ALP) activities

There was significant increase (p < 0.05) in the serum and liver γGT activities of the rats treated with sodium arsenite, as compared with the negative control rats treated with distilled water alone (group A vs B, Figure 1). We observed that treatment of the animals with the extracts 14 days before or just after sodium arsenite administration (groups E, F and G) significantly (p < 0.05) reduced mean liver and serum γGT activities when compared with group administered the toxin alone (group B). Treatment with the extract of *O. basilicum* alone resulted in higher liver and serum γGT as compared with the negative control group. However, there are no significant differences (p > 0.05) in the observed γGT values within the groups of rats treated with *O. basilicum* aqueous or ethanolic extract alone (group C or D) and the negative control group (group A). Serum ALP activities of the treated rats also showed trends similar to what was observed for the γGT activities (Figure 2).

![Fig. 1:](image-url)

Leaf extracts of *Ocimum basilicum* suppressed sodium arsenite-induced liver and serum γ-glutamyl transferase (γGT) activities in the Wistar rats. A, group of rats given distilled water only (this is the negative control group); B, group of rats given sodium arsenite (this is the positive control group); C, animals in this group were given aqueous extract of *O. basilicum* leaves alone; D, animals in this group were given ethanolic extract of *O. basilicum* leaves only; E, group pre-treated with aqueous extract of *O. basilicum* leaves before sodium arsenite; F, group pre-treated with ethanolic extract of *O. basilicum* leaves before sodium arsenite; G, animals were given sodium arsenite same time as aqueous extract of *O. basilicum* leaves. Values are Mean ± SD; n=5. *Significantly different from the negative control; # significantly different from positive control group (p < 0.05).
Leaf extracts of *Ocimum basilicum* suppressed sodium arsenite-induced serum alkaline phosphatase (ALP) activities in the treated rats. A, group of rats given distilled water only (this is the negative control group); B, group of rats given sodium arsenite (this is the positive control group); C, animals in this group were given aqueous extract of *O. basilicum* leaves alone; D, animals in this group were given ethanolic extract of *O. basilicum* leaves only; E, group pre-treated with aqueous extract of *O. basilicum* leaves before sodium arsenite; F, group pre-treated with ethanolic extract of *O. basilicum* leaves before sodium arsenite; G, animals were given sodium arsenite same time as aqueous extract of *O. basilicum* leaves. Values are Mean ± SD; n=5. *Significantly different from the negative control; # significantly different from positive control group (p < 0.05).

**Attenuation of sodium arsenite-induced serum alanine (ALT) and aspartate aminotransferase (AST) activities by leaf extracts of *O. basilicum***

Experimental rats administered sodium arsenite (i.p) have elevated serum alanine (282.6 %) and aspartate aminotransferase (325.1 %) activities as compared with the negative control group (Figure 4). Treatments of the animals with aqueous or ethanolic extract of *O. basilicum* before the administration of sodium arsenite resulted in the attenuation of the sodium arsenite induction of these serum enzymes: ALT (167.7 % and 157.8 %), AST (173.5 % and 164.2 %) for the aqueous and ethanolic extracts treatment respectively before sodium arsenite administration expressed as percentage of the negative control. There were no significant (p > 0.05) differences in the mean enzyme activities between the group pre-treated with the ethanolic extract before sodium arsenite (group F) and the group administered the extract just after the toxin (group G) or between the groups given the aqueous or ethanolic extract pre-treatments (groups E and F).

**Effect of sodium arsenite and leaf extracts of *O. basilicum* on glutathione-S-transferase (GST) in rats**

Experimental rats treated with sodium arsenite have mean liver GST activities that were significantly lower (p < 0.05) than the observed mean value in the negative control rats treated with distilled water alone (groups B vs A; Figure 4). On the other hand, the group of rats treated with *O. basilicum* leaf extracts alone (groups C and D) recorded mean liver GST values that were significantly higher (p < 0.05) than the negative control group (A). Treatment of the animals with aqueous or ethanolic leaf extracts of *O. basilicum* before or just after the administration of sodium arsenite resulted in mean GST values that are similar to those obtained when the animals received the distilled water alone.
Fig. 3: Attenuation of sodium arsenite-induced serum alanine and aspartate aminotransferase activities by leaf extracts of *O. basilicum*. **A**, group of rats given distilled water only (this is the negative control group); **B**, group of rats given sodium arsenite (this is the positive control group); **C**, animals in this group were given aqueous extract of *O. basilicum* leaves alone; **D**, animals in this group were given ethanolic extract of *O. basilicum* leaves only; **E**, group pre-treated with aqueous extract of *O. basilicum* leaves before sodium arsenite; **F**, group pre-treated with ethanolic extract of *O. basilicum* leaves before sodium arsenite; **G**, animals were given sodium arsenite same time as aqueous extract of *O. basilicum* leaves. Values are Mean ± SD; n=5. *Significantly different from the negative control; # significantly different from positive control group (p < 0.05).

Fig. 4: Effect of sodium arsenite and leaf extracts of *O. basilicum* on glutathione-S-transferase activities in the Wistar rats. **A**, group of rats given distilled water only (this is the negative control group); **B**, group of rats given sodium arsenite (this is the positive control group); **C**, animals in this group were given aqueous extract of *O. basilicum* leaves alone; **D**, animals in this group were given ethanolic extract of *O. basilicum* leaves only; **E**, group pre-treated with aqueous extract of *O. basilicum* leaves before sodium arsenite; **F**, group pre-treated with ethanolic extract of *O. basilicum* leaves before sodium arsenite; **G**, animals were given sodium arsenite same time as aqueous extract of *O. basilicum* leaves. Values are Mean ± SD; n=5. *Significantly different from the negative control; # significantly different from positive control group (p < 0.05).
DISCUSSION

Reduced glutathione (GSH), $\gamma$-glutamyl transferase, $(\gamma GT)$ and glutathione-S- transferase (GST) play important role in the protection of cells against carcinogenic chemicals (Locigno and Castronovo, 2001). In addition, elevation in the level of the enzymes, $\gamma$GT and alkaline phosphatase (ALP) has emerged as an index of a liver lesion (Lum and Gambino 1972, Friedman et al. 1996). Arsenicals are becoming environmental contaminant of significance. In fact, long term exposures to arsenic through drinking wells’ water with high concentration of arsenic have been associated with the aetiology of some cancers (Chen et al. 1992, Chow et al. 1997). Rats treated with sodium arsenite (positive control group B) have mean liver and serum $\gamma$GT and serum ALP at levels that are significantly higher than the value observed for the negative control group (group A). Previous studies in our laboratories showed that sodium arsenite induced $\gamma$GT activity in laboratory animals (Odunola et al. 2008, Gbadegesin et al. 2009). The elevation in the level of the two enzymes, $\gamma$GT and ALP, is an indication of a liver lesion (Lum and Gambino 1972, Ideo et al. 1972). On the other hand, we found that the aqueous and ethanolic leaf extracts of *O. basilicum* attenuates the sodium arsenite-induced enzymes. Treatment with leaf extracts of *O. basilicum* before or just after administration of sodium arsenite significantly ($p < 0.05$) reduced mean liver and serum $\gamma$GT and ALP activities when compared with groups administered only sodium arsenite, supporting the presence of hepatoprotective components in the extracts against the hepatotoxicity of the sodium arsenite.

In addition, we found that sodium arsenite-induced higher level of serum ALT (282.6 %) and AST (325.1 %) in the treated rats as compared with the negative control group that received distilled water alone. This is consistent with findings with other hepatotoxins (Hsiao et al. 2001, Ritter et al. 2004). Treatment with leaf extracts of *O. basilicum* before or after sodium arsenite led to reduction in the level of sodium arsenite-induced ALT and AST activities.

Furthermore, treatment of the experimental rats with the *O. basilicum* extracts led to enhanced level of hepatic GST compared with the experimental group with distilled water alone. *Ocimum basilicum* extracts treatment also reduced the decreased GST activity observed with sodium arsenite treatment to the level similar to the negative control group. The findings here are similar to the report about extract from *Ocimum sanctum* by Prashar et al. (1994). This suggests that the extracts contain reduced glutathione replenishing ability. The hepatoprotective effects of *Ocimum basilicum* (sweet basil) extracts may be linked to their antioxidant activities. Further studies are on to elucidate the mechanism by which *O. basilicum* extracts is able to protect against sodium arsenite induced hepatotoxicity in the experimental animals. The findings here support the growing evidence that vegetables, spices and fruits exhibit protective effects against chemicals and toxins (Prashar et al. 1994, Biswas et al. 1999, Karthikeyan et al. 1999, Rastogi et al. 2007).

REFERENCES


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associated with chronic exposure to arsenic. *Br J Cancer*, 75, 1708-10.


