Estimation of *Lippia alba* antioxidants' activities in ochratoxin A intoxicated albino rats

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**Abstract.** The participation of antioxidants in body cellular processes cannot be over-emphasized as they protect and reduce oxidative damage within the cell and its surrounding membranes. These chemical substances act on free radicals by donating free electrons to unstable radicals thereby haltering their capacity to steal electrons. The capacity of *Lippia alba* extract to ameliorate antioxidant levels in ochratoxin A intoxicated albino rats was investigated. Sixty albino rats divided into two sets were used in the study. Animals were administered a single dosage of 2mg ochratoxin A per 250 mg bw of animals, followed by the administration of the extract at 300 mg per kg body weight by gavage. Results showed that intoxication with 2 mg OTA/250 g bw reduced the glutathione levels in the two sets by 55.3 %, 47.1 %, 59.3 %, and 65.9 % for pre-treated (kidney), post-treated (kidney), pre-treated (liver), and post-treated (liver) respectively. Superoxide dismutase values obtained highest recovery in group 5 pre-treated animals with increased values in the kidney and liver (51.37 ± 2.13 U/mg protein and 23.52 ± 1.78 U/mg protein respectively). Malondialdehyde values reduced to 42.78 ± 4.13 mol/g kidney tissue (pre-treated) and 53.60 ± 2.96 mol/g kidney tissues (post-treated set) which was a 25.1 % difference between the pre-treated and post-treated sets. Conclusively, pre-treatment with *L. alba* increased antioxidant levels and it also reduced unstable radicals formed due to oxidative damage caused by xenobiotic ochratoxin A as seen in the results obtained. The extract thus improves the activities of antioxidant malondialdehyde, glutathione, SOD, and glutathione s-transferase.

**Keywords:** Antioxidants, radicals, *Lippia*, intoxication

**Introduction**

Antioxidants are substances capable of neutralizing free radicals and reducing oxidative damage within the cell and surrounding membrane. These chemical substances, enzymes, elements, vitamins have the capacity to mop up free radicals and prevent them from causing cell damage [1]. They neutralize toxic radicals by bonding them through the donation of their own electrons and in the process altering the carbon-stealing reaction [2]. Sen et al. [1] and Jacob [2] classified antioxidant into endogenous enzymatic antioxidants, non-enzymatic antioxidants, metabolic and nutrient antioxidants, and metal binding proteins. Antioxidant production by the human body decreases with age [3]; though the body produces only endogenous antioxidants which are classified into enzymatic and non-enzymatic groups in response to the destructive consequence of ROS. The enzymatic antioxidant consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), while the non-enzymatic systemare the vitamin E, vitamin C and reduced glutathione (GSH) [1, 2, 4].

Free radicals are chemical substances produced in the body by the oxidative metabolism of food substances to obtain energy with capacity to induce disease conditions [1]. These are molecules or its fragment with one or more unpaired electrons formed through series of reduction-oxidation reactions with capacity for independent existence and not very stable [5, 6, 7]. Molecules such as RNA, DNA and protein enzymes are all susceptible to oxidative damage caused by free radicals within the cell.

Glutathione GSH a non-enzymatic antioxidant produced in the liver that produces other antioxidants, while also detoxifying toxic substances; and strengthening the immune system [8, 9]. It is a co-factor of glutathione peroxidase (GPx) that help in the elimination of peroxides formed during lipid peroxidation and ROS [1]. Superoxide dismutase (SOD) protects the cell from superoxide toxicity, act against ROS by scavenging superoxide radicals to hydrogen peroxide Malondialdehyde (MDA) is the decomposition product of polyunsaturated fatty acids of

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biological membranes and its increase shows the extent of membrane lipid peroxidation [10, 11].

**Materials and Methods**

**a) Preparation of albino rat model**

Albino rats were obtained and kept in conformity with laid down policy of “Guide for care and use of laboratory animals in research and teaching” by the National Academy of Science, published by the National Institute of Health (NIH) publication 86-23 [1985 revised].

**b) Experimental design**

Sixty (60) animals were employed in the study. The animals were divided into two (2) sets of 30 animals with each set sub-divided into six groups of 5 animals each according to the methods of Oroke et al. [12]. The animals were allowed to acclimatize for two weeks (14 days) before the start of the experiment. Animals in Set 1: *Lippia alba* pre-treated set were administered crude *Lippia alba* extract at 300 mg/kg body weight for a period of three weeks before intoxication depending on the group to build the immunity of the animals. Group 1: Negative Control – Animals were given DMSO alone, Group 2: Positive Control – Animals were treated with 300 mg *L alba* per kg body weight, Group 3: Animals were treated with 2 mg ochratoxin A per 250 mg bw of animals only, Group 4: Animals were treated with 1 mg ochratoxin A per 250 mg bw of animals only.

Group 5: Animals were treated with 2 mg OTA/250 g bw + 300 mg *L alba* per kg body weight, Group 6: Animals were treated with 1 mg OTA/250 g bw + 300 mg *L alba* per kg body weight, while Set 2: *Lippia alba* post-treated animals were administered extract at 300 mg/kg body weight after intoxication depending on the group. The animals 3 h after intoxication with observation for behavioral changes were treated with *Lippia alba* extract based on body weight as in Set 1.

**c) Extracts and ochratoxin A preparation and administration to albino rats**

Ten milligram per milliliter (10 mg/mL) extract was prepared and administered to animals at concentration of 300 mg/kg body weight. Extract administration was done every three days at 0700 hours in the morning by oral administration. Administration of ochratoxin A (Trilogy Analytical Laboratory; Product Number TSL-504) was through intraperitoneal route after dissolving the toxin in sterile Dimethyl sulphoxide (DMSO) and volume and dose adjusted to animal weight.

**d) Organ collection and preparation of liver and kidney homogenates**

Seven after intoxication, animals were euthanized and subsequently sacrificed by cervical dislocation after which the liver and kidney were removed, washed with water and blotted with filter paper then weighed individually. Employing the modified methods of Noori et al. [13], organs were separately perfused in saline solution then homogenized in chilled potassium chloride (1.17%). The homogenates were centrifuged to remove debris after which it was further centrifuged at 10,500 g for 20 min at 4°C to obtain post mitochondrial supernatant needed for antioxidant assay. The kidney homogenates (1:10 w/v) were prepared by using a 100 mmol KCl buffer (pH 7.0) containing EDTA 0.3 mM, and the homogenates were centrifuged at 600 g for 60 minutes at 4°C and the supernatant used for assay.

**e) Estimation of Malondialdehyde (MDA)-Lipid peroxidation**

Lipid peroxidation level was estimated using the modified methods of Nayanatara et al. [14] and Kartha and Krishnamurthy [15]. Five millilitres of freshly prepared homogenate was incubated at 39°C for 30 min along with “the blank” in a water bath in a separate conical flask. After incubation, 1 mL was added to the tube containing 1.5 mL of 20% cold trichloroacetic acid (TCA) and then centrifuged for 10 min. After centrifugation, 2 mL of the supernatant fluid was added 2 mL of 0.7% thiobarbituric acid (TBA) and kept in the boiling water bath for 10 min. The development of pink color was measured at 535 nm by using spectrophotometer.

**f) Estimation of Superoxide Dismutase (SOD)**

Superoxide dismutase (SOD) activity was measured using assay kit (Cayman, MI, USA) according to manufacturer's instructions. This kit utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was defined as the amount of enzyme needed to produce 50% dismutation of superoxide radical.

**g) Estimation of Glutathione-S-Transferase (GST)**

Glutathione-S-transferase (GST) activity was assayed according to the modified methods of Erejuwa et al. [16, 17]. To 2 mL of 0.3 M potassium phosphate buffer (pH 6.35), 75 μL of 30 mM CDNB solution, 725 μL of distilled water was added 0.1 mL of the organ homogenate in a test tube. The test tube was vortexed and incubated at 37°C for 10 min. After incubation, the reaction was initiated by the addition of 100 μL of 30 mM reduced glutathione solution and measured using a spectrophotometer at 340 nm and recorded every 30 seconds for 4 minutes.

**h) Estimation of Glutathione (GSH)**

Glutathione activity was determined by the procedure of Noori et al. [13]. The assay solution contained 10% BSA (bovine serum albumin), 50 mM potassium phosphate buffer (pH 7.6), 2 mM NADPH, and 20 mM oxidized glutathione. Absorbance at 340 nm was recorded at a temperature of 25°C. The activity was calculated using the molar coefficient for NADPH of 6.22 μmol⁻¹ x cm⁻¹ and expressed in U/g of tissue.

**i) Statistical analysis**

The result of antioxidants levels were expressed as means and Standard Error of Means (SEM). Analysis of Variance was obtained and the means were separated using
Results

i) Malondialdehyde levels in kidney and liver of *Lippia alba* treated rats

Malondialdehyde levels as shown in Table 1 obtained values ranging between 9.90 ± 0.68 mol/g kidney tissue as the lowest (group 2) and 27.60 ± 4.37 mol/g kidney tissue highest in group 3 of the pre-treated set. MDA values in group 1 animals fell within the normal range then increased by 138.3 % and 158.4 % in the pre-treated and post-treated sets respectively. The values then decreased by 78.3 % and 28.7 % as seen in group 5 animals, though the effects were higher in the pre-treated set (16.60 ± 1.21 mol/g kidney tissue). In kidneys the pre-treated and post-treated sets, values were only significantly different between animals in group 3 at p<0.05. Malondialdehyde values in the liver of the animals recorded about 100 % increases when compared with animals in group 3 of each set. The result showed that the extract decreased malondialdehyde values in the pre-treated and post-treated sets as seen in groups 5 and 6 respectively.

ii) Glutathione levels in kidney and liver of *Lippia alba* treated rats

Glutathione levels as shown in Table 2 obtained values that increased in group 2 animals administered with *Lippia alba* only in the pre-treated and post-treated sets when compared with the negative control groups administered DMSO only. The result showed administration of 2 mg OTA/250 g bw reduced the GSH values across the two sets by 55.3 %, 47.1 %, 59.3 %, and 65.9 % for pre-treated (kidney), post-treated (kidney), pre-treated (liver), and post-treated (liver) respectively. The result also showed that GSH in kidney of group 3, 4, 5 and 6 of set 1 animals were significantly different from groups 1 and 2, while the same is applicable to kidney GSH values in set 2 animals at p<0.05. GSH values in the pre-treated and post-treated sets were significantly different in groups 1 and 2 from the rest of the groups at p<0.05.

iii) Superoxide dismutase activities in kidneys and livers of *Lippia alba* treated rats

Table 3 presented increases in superoxide dismutase (SOD) values as obtained in group 2 animals administered with 300 mg *Lippia alba* per kg body weight across the two sets, but was contrasted by lower values obtained when group 3 animals in the two sets were administered 2 mg and 1 mg ochratoxin A per 250 g body weight respectively in the kidneys and livers of the animals. Superoxide dismutase (SOD) values were significantly different within the groups except in (groups 4 and 5 of kidney: pre-treated set), (groups 5, 6, and 3, 4: liver pre-treated set), and (groups 3, 4, and 5, 6 in the *Lippia alba* post-treated set).

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Table 1: Malondialdehyde Levels in the Kidneys and Livers of Rat Intoxicated with Ochratoxin A

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Set 1: <em>L. alba</em> Pre-treated</th>
<th>Set 2: <em>L. alba</em> Post-treated</th>
<th>MDA in kidney (mol/g tissue)</th>
<th>MDA in liver (mol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp 1: Negative Control (DMSO only)</td>
<td>12.42 ± 1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.84 ± 0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.89 ± 3.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.5 ± 2.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gp 2: Positive Control (300 mg <em>L. alba</em>/kg bw)</td>
<td>9.90 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.00 ± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.04 ± 1.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.00 ± 2.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gp 3: 2 mg OTA/250 g bw</td>
<td>27.60 ± 4.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.60 ± 2.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.73 ± 3.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.30 ± 3.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gp 4: 1 mg OTA/250 g bw</td>
<td>22.40 ± 3.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.20 ± 2.63&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65.40 ± 5.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70.20 ± 2.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gp 5: 2 mg OTA/250 g bw + 300 mg/kg bw <em>L. alba</em></td>
<td>16.60 ± 1.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.60 ± 2.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>42.78 ± 4.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>53.60 ± 2.96&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gp 6: 1 mg OTA/250 g bw + 300 mg/kg bw <em>L. alba</em></td>
<td>18.50 ± 0.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.00 ± 1.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37.96 ± 4.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.01 ± 2.66&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; Values with different superscripts within the column are significantly different at p<0.05 by Tukey HSD test.

Table 2: Glutathione Levels in Rat Organs Intoxicated with Ochratoxin A

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Set 1: <em>L. alba</em> Pre-treated</th>
<th>Set 2: <em>L. alba</em> Post-treated</th>
<th>GSH in kidney (ug/g tissue)</th>
<th>GSH in liver (ug/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp 1: Negative Control (DMSO only)</td>
<td>6.66 ± 0.34&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>6.80 ± 0.81&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>11.66 ± 0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.90 ± 0.56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gp 2: Positive Control (300 mg <em>L. alba</em>/kg bw)</td>
<td>7.80 ± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.40 ± 0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.60 ± 1.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.57 ± 0.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gp 3: 2 mg OTA/250 g bw</td>
<td>2.98 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.60 ± 0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.75 ± 0.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.40 ± 0.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gp 4: 1 mg OTA/250 g bw</td>
<td>2.92 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.70 ± 0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.97 ± 0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.00 ± 0.45&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gp 5: 2 mg OTA/250 g bw + 300 mg/kg bw <em>L. alba</em></td>
<td>5.14 ± 0.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.44 ± 1.86&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.20 ± 0.49&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.30 ± 0.54&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gp 6: 1 mg OTA/250 g bw + 300 mg/kg bw <em>L. alba</em></td>
<td>5.28 ± 0.57&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.02 ± 0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.30 ± 0.89&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.95 ± 0.47&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; Values with different superscripts within the column are significantly different at p<0.05 by Tukey HSD test.

Tukey’s Kramer post hoc test at p ≤0.05.
**TABLE 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Set 1: L. alba Pre-treated</th>
<th>Set 2: L. alba Post-treated</th>
<th>Set 1: L. alba Pre-treated</th>
<th>Set 2: L. alba Post-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST in kidney (mg/mL)</td>
<td>SOD in kidney (U/mg tissue)</td>
<td>GST in liver (U/mg tissue)</td>
<td>SOD in liver (U/mg tissue)</td>
<td></td>
</tr>
<tr>
<td>Gp 1: Negative Control (DMSO only)</td>
<td>67.12 ± 2.64a</td>
<td>69.29 ± 1.26b</td>
<td>32.62 ± 0.51c</td>
<td>30.56 ± 1.11d</td>
</tr>
<tr>
<td>Gp 2: Positive Control (300 mg Lippia alba/kg bw)</td>
<td>82.61 ± 1.02a</td>
<td>81.59 ± 1.55b</td>
<td>40.82 ± 1.16c</td>
<td>42.36 ± 1.80d</td>
</tr>
<tr>
<td>Gp 3: 2 mg OTA/250 g bw</td>
<td>24.46 ± 2.48a</td>
<td>34.47 ± 1.42b</td>
<td>13.30 ± 0.91c</td>
<td>13.20 ± 1.62d</td>
</tr>
<tr>
<td>Gp 4: 4 mg OTA/250 g bw</td>
<td>28.16 ± 2.73a</td>
<td>38.61 ± 1.79b</td>
<td>16.20 ± 0.58c</td>
<td>15.49 ± 0.71d</td>
</tr>
<tr>
<td>Gp 5: 2 mg OTA/250 g bw + 300 mg/kg bw L. alba</td>
<td>51.37 ± 2.13a</td>
<td>44.53 ± 1.61b</td>
<td>23.52 ± 1.78c</td>
<td>23.97 ± 1.04d</td>
</tr>
<tr>
<td>Gp 6: 1 mg OTA/250 g bw + 300 mg/kg bw L. alba</td>
<td>53.28 ± 0.66a</td>
<td>46.20 ± 1.28b</td>
<td>26.49 ± 1.44c</td>
<td>27.36 ± 0.85d</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; Values with different superscripts within the column are significantly different at p<0.05 by Tukey HSD test

**TABLE 4**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Set 1: L. alba Pre-treated</th>
<th>Set 2: L. alba Post-treated</th>
<th>Set 1: L. alba Pre-treated</th>
<th>Set 2: L. alba Post-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST in kidney (mg/mL)</td>
<td>GST in liver (mg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gp 1: Negative Control (DMSO only)</td>
<td>27.00 ± 1.58a</td>
<td>28.80 ± 0.58b</td>
<td>48.37 ± 1.07c</td>
<td>46.55 ± 1.56d</td>
</tr>
<tr>
<td>Gp 2: Positive Control (300 mg Lippia alba/kg bw)</td>
<td>35.24 ± 1.22a</td>
<td>34.00 ± 1.18b</td>
<td>64.55 ± 2.33c</td>
<td>65.80 ± 3.68d</td>
</tr>
<tr>
<td>Gp 3: 2 mg OTA/250 g bw</td>
<td>14.88 ± 0.88a</td>
<td>16.50 ± 0.50b</td>
<td>20.38 ± 3.03c</td>
<td>24.07 ± 2.05d</td>
</tr>
<tr>
<td>Gp 4: 1 mg OTA/250 g bw</td>
<td>15.08 ± 1.15a</td>
<td>17.04 ± 0.62b</td>
<td>23.34 ± 1.05c</td>
<td>23.89 ± 2.72d</td>
</tr>
<tr>
<td>Gp 5: 2 mg OTA/250 g bw + 300 mg/kg bw L. alba</td>
<td>18.60 ± 0.51a</td>
<td>19.80 ± 0.58b</td>
<td>36.31 ± 2.40c</td>
<td>28.37 ± 1.81d</td>
</tr>
<tr>
<td>Gp 6: 1 mg OTA/250 g bw + 300 mg/kg bw L. alba</td>
<td>21.00 ± 0.71a</td>
<td>21.57 ± 0.50b</td>
<td>40.49 ± 2.49c</td>
<td>29.61 ± 0.98d</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; Values with different superscripts within the column are significantly different at p<0.05 by Tukey HSD test

**iv) Glutathione S-transferase levels in kidney and liver of L. alba treated rats**

Glutathione S-transferase (GST) values in kidneys of the two albino rats sets reduced by over 42% (L. alba pre-treated) and 48% (L. alba post-treated) when group 3 animals were differently administered 2 mg OTA per 250 g bw (Table 4). The results were opposed by group 2 animals with 30.5% (L. alba pre-treated) and 18.1% (L. alba post-treated) increment over the negative control when the animals were treated with L. alba crude extract. Pre-treated and post-treated animals’ results showed that improvement recorded in the values (groups 5 and 6) were significantly different at p<0.05. GST values of the liver tissue obtained the same trend as seen in groups 5 and 6 of the pre- and post-treated sets. Values obtained between the two (group 5 and group 6) were not significantly different from each other but significantly different from other groups within the set at p<0.05.

**DISCUSSION**

Ochratoxin A induces ROS production which promotes oxidative damage, and thus interferes with glycolytic and glycolgenetic pathways, while promoting gluconeogenesis, glycogenolysis, and membrane lipid peroxidation. Malondialdehyde values in the animal kidneys in groups intoxicated with ochratoxin A only (both sets) were high compared to values obtained for the control groups and group administered 300 mg Lippia alba/kg bw. Ochratoxin A being a xenobiotic compound enhances the production of free radical and in the process create oxidative stress state in animals which might eventually cause damage to macromolecules [18]. Oxidative stress state results in excessive production of reactive oxygen species (ROS) which leads to lipid peroxidation characterized by malondialdehyde; a decomposition product of polyunsaturated fatty acid of membranes [10, 11].

High above normal MDA values obtained in albino groups intoxicated with 1 mg and 2 mg OTA per 250 g bw respectively showed that the administered ochratoxin A generated reactive oxygen species (ROS) which might have led to the leaching of the cellular components of the kidney and subsequent lipid peroxidation as seen in the result obtained. Chakraborty and Verma [19] proposed that ochratoxin A increases cell permeability to Ca²⁺ and enhances cellular concentration of Ca²⁺. In addition, Hoehler et al. [20] explained that ochratoxin A breaks down oxidative phosphorylation which results in electron leakage within the cell thus altered or increased MDA values. The chemo-preventive activities of L. alba is seen in lower levels of MDA in the liver and kidney of pre-treated groups when compared to post-treated set (Group 5 and 6). Lippia alba contains limonene, perillyl alcohol, carvone, and geraniol - all terpenoid which confers protective properties on the plant [21]. Citral present in the plant had the capacity to inhibit CYP2B60 hydroxylase activity and suppress oxidative stress through the induction of endogenous antioxidant protein as reported by Seo et al. [22] and Nakamura et al. [23].
Glutathione level in body organs is an indicator of cell's ability to deal with toxicity challenges [24]. Results presented in this study showed that significant reductions were noticed in GSH levels in ochratoxin A treated animal models of Groups 3 and 4 for the sets. Findings showed that ochratoxin A brought about reduction in GSH levels in animal models which agreed with the findings of Abdel-Aziz et al. [25], Abdel-Wahhab et al. [26], and Chakraborty and Verma [19]. Abdel-Wahhab et al. [26] reported that reduction in GSH activities likely resulted from electrophilic neutralization of ochratoxin A radicals and other reactive oxygen species formed by ochratoxin A. Results of the increasing levels of GSH in *L. alba* treated rats (Groups 5 and 6) agreed with the result presented by Young et al. [2000, 27] and Analikumar et al. [2001, 28] who both suggested that the presence of polyphenol confers protection against oxidative stress leading to a corresponding increase in the level of GSH (Noori et al., 2009, 13).

Superoxide dismutase (SOD) activities showed decreasing values when comparison is done with the control group that obtained higher values. These results are in agreement with Abdel-Aziz et al. [25] and Abdel-Wahhab et al. [26] who proposed that the decreasing SOD values obtained might have resulted from damages to the cell of the kidney and liver as suggested by Doorten et al. [2004, 29]. Dismutation of superoxide radicals into molecular oxygen or hydrogen peroxide by SOD decreases its levels in the kidney and liver. SOD activities in the *L. alba* pre-administered animal groups showed improved results in the kidney than in the liver. *Lippia alba* contains vitamin C which is a potent water soluble antioxidant with the ability to scavenge free reactive oxygen species and thus protect cells against oxidative damage (Pavan et al., 2007, 30). The vitamin promotes its activities by donating electron to hydroxyl (OH) ion and superoxide radicals thereby inactivating their reactivity and in the process restoring SOD levels back to within the normal range. Slight differences were however noted in SOD levels of livers in groups 5 and 6 animals of the pre- and post-treated sets showing that the extract did not advance protection and healing as it did in the kidney cells. Manikandar and Devi [31] also reported the presence of fat soluble vitamin E as an antioxidant present in *Lippia alba* with the capacity to protect membrane fatty acids from peroxidation. The vitamin hinders peroxidyl mediated chain reaction and also scavenge SO in the lipid membrane.

Results of findings presented in this study on glutathione S transferases (GST) agrees with earlier studies by Chakraborty and Verma [19], Soyoz et al. [32], and Verma and Chakraborty [33] who obtained significantly lower glutathione S transferase enzyme in ochratoxin A administered animals. Chakraborty and Verma [19] in their presentation proposed that the reduction in the GST level simultaneously brings about increasing activities of oxygen species. Reduced GST levels might be adduced to the toxicity of ochratoxin A and its capability to cause disruption to the membrane integrity of the liver and kidney cell respectively. Albino rats in Set 1 administered *Lippia alba* only (Group 2) obtained elevated levels of antioxidant GST compared to the control group within the same set. This might be attributed to overproduction of GST in response to introduction of foreign substances into the cells which is subsequently brought down by the activities of glutathione S transferase conjugates with functionalized P450 metabolites and vitamin C which decreases the activities of several cytochrome P450 isoenzyme thus increasing GST levels in *Lippia alba* treated rats (Sorrenti et al. [34]). Antioxidants in the plant which aided in prevention of formation of ROS and free radicals include selenium and zinc. These brought about amelioration within the cells by altering apoptosis induced by ochratoxin A in mice liver as reported by Sorrenti et al. [4] and Zheng et al. [35]. Chies et al. [36] explained that pre-treatment with *L. alba* prevents formation of malondialdehyde that promotes genotoxicity from OTA-DNA adduct and hinders induced SOD and CAT phenomena that result in the production of free oxidative reactive species within the cell membrane.

**Conclusion**

The study proved reasonably that *Lippia alba* had the potential to alter ochratoxin A induced toxicities in the form of ROS and free radical production which showed in the activities of antioxidants produced in the liver and kidney. Extract of the plant significantly restore or either ameliorate the activities of the antioxidants.

**Conflict of Interest**

The authors declare no conflicts of interest.

**References**


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