

## Effect of *Lippia alba* in the Detoxification of Ochratoxin A in Albino Rats

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### Abstract

The problems and associated challenges of mycotoxins' contaminations are on the increase, encouraged by favorable climatic conditions which results from greenhouse effect and global warming. Ochratoxin A (OTA), a mycotoxin reduces food quality, causes cancerous conditions and ochratoxicosis in man and animals thus making it imperative to find a cost-effective and non-toxic method to prevent contamination of stored agricultural produce and crops. Investigation was carried out to determine the ability of *Lippia alba* to detoxify ochratoxin A (OTA) in albino rats. Rats (120) weighing between 250 and 300g were divided into *L. alba* pre- and post-treated sets. Seven days after intraperitoneal OTA intoxication, animals were euthanized and blood was harvested for serum enzymes and quantification of biochemical parameters. Analysis of Variance was obtained and the means of blood parameters were separated using Tukey's Kramer post hoc test at  $p \leq 0.05$ . The result obtained showed that treatment of animals with OTA altered weight values with weight increase observed in pre-treated animals. The administration of *L. alba* restored OTA altered blood urea level from 5.5 to 6.95 mg/dL, alkaline phosphatase (35 - 121 U/L), and aspartate aminotransferase from 205.3 to 187.3 U/L. The results presented evidences that *L. alba* demonstrated potency to restore and ameliorate OTA altered biochemical indices that resulted from ochratoxin A's disruption of hepatocytes and renal components. The study provided evidences of the therapeutic capabilities of *Lippia alba* to heal and protect kidney and liver in treated animals.

**Keywords:** Ochratoxin A; *Lippia*; Detoxify; Parameters; Intraperitoneal

### Introduction

Negative interaction between microorganisms and other higher organisms put the later in compromised positions that lead to infections and disease conditions. Infection of hosts by microorganisms induces the release of toxins and subsequent disruption of cellular constituents of the host system. The need to explore plant parts for healing and therapeutic activities had never been more urgent [1,2] more so that, plants are abundant, renewable, user-friendly and environmentally safe [3]. Phytochemicals from plants contain an array of important active components [4], which vary depending on their growing conditions, varietal differences, and age at harvest, extraction methods, and storage conditions [2]. Development and formulation of phytochemicals with capacity to remove, deactivate, or detoxify mycotoxins [5] is a continuum; and when available should be acceptable, cost-effective, and without residual toxicities.

Ochratoxin A, a metabolite produced by filamentous *Aspergillus* and *Penicillium* species in the tropics and temperate regions of the world are weak organic acids classified by the National Toxicology Program (NTP) as "reasonably anticipated to be a human carcinogen"

[6], while COT [7] classified it as a genotoxic carcinogen. The World Health Organization (WHO) and Food and Agriculture Organization (FAO) set provisional maximum intake of 100 ng/kg body weight, while the Scientific Committee on food of EU set 5 ng/kg body weights as the provisional maximum intake [8]. OTA has half-life of 35 days in human [9] as a result of its capacity to covalently bind to serum protein particularly albumin [10].

Reports abound of plant materials reported to detoxify other mycotoxins, but little information is available on detoxification of ochratoxin A. Mossini, *et al.* [11] reported that *Azadirachta indica* extracts inhibited ochratoxin A production by fungi species *Penicillium verrucosum* and *P. brevicompactum*; piperine and piperlongumine compounds in *Piper longum* plant inhibited ochratoxin A synthesis by *Aspergillus auricomus*, *A. sclerotiorum* and isolates of *A. alliaceus*, while garlic and laurel plants prevented ochratoxin A production in *A. ochraceus*. Reports showed that at different concentration, plant exhibited varying capacities at preventing synthesis of OTA; Cinnamon (3%), anis (4%), oil of thyme and cinnamon (3000 ppm) [12,13]. The use of *L. alba* results from its potential to inhibit *A. niger*, *A. niger* aggregate, *A. carbonarius*, *A. terreus*, *A. ochraceus* which are all producers of OTA [14,15].

Toxicity confers liver injury which is a mix of different metabolic process dysfunctions to include DNA damage cum blocked protein synthesis, increased catabolism involving phenylalanine, leakage of damaged tissues, lipid peroxidation and mitochondrial respiration interference [16,17]. The authors proposed that increased level of enzymes in the serum is a function of enzyme leakage from the liver cystole, enzyme release by tumor invaded tissues, or the resultant effects of tumor on the surrounding tissues. Alanine aminotransphosphate (ALT) is present in the cytosol of the liver, mitochondria, tissues, liver, heart, skeletal muscles, kidneys, brain, and the white and red blood cells. ALT and Aspartate aminotransferase (AST) are markers of liver injury [18]. Any small elevation in the level of ALT in the plasma calls for concern [19,20], as the marker is specific for hepatic injury and is found mainly in the cytosol of the hepatocyte of liver in high concentration. Injury, stress conditions, and cellular damage all increase ALT values in the serum.

## Materials and Methods

### Collection of albino rats for ochratoxicity study

One hundred and twenty (120) albino rats weighing 250 - 300g obtained from Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko were used for the study. The animals were kept under 12h light and 12h dark conditions were provided animal feed and water ad libitum at  $25 \pm 2^{\circ}\text{C}$  till the end of the study. Animal feeds were prepared by Grand cereals limited, a subsidiary of UAC of Nigeria plc, Jos, Nigeria. The feed was confirmed to be free of ochratoxin A before use. Maintenance of the animals was in conformity with the laid down policy of the Institute of Health (NIH) (1985 revised) which deals with the use of laboratory animals in research.

### Experimental design

The animals were divided into two (2) sets of 60 animals each; each set was further divided into six groups of ten animals thus; *Lippia alba* pre-treated administered crude aqueous *Lippia alba* extract at 300 mg/kg body weight for a period of three weeks before intoxication with ochratoxin A. The idea was to build the immunity of the animals; and *Lippia alba* post-treated administered extract after intoxication. The animals were observed for behavioral changes 3 h after OTA treatments.

Group 1: Negative Control – Animals treated with 10% DMSO alone

Group 2: Positive Control – Animals administered 300 mg *Lippia alba* per kg body weight

Group 3: Animals administered 2 mg ochratoxin A per 250g bw of animals only

Group 4: Animals administered 1 mg ochratoxin A per 250g bw of animals only

Group 5: Animals administered 2 mg OTA per 250g bw + 300 mg *Lippia alba* per kg bw

Group 6: Animals administered 1 mg OTA per 250g bw + 300 mg *Lippia alba* per kg bw

Animals were allowed to acclimatize for two weeks (14 days) before the start of the experiment.

**Body weight of treated albino rats**

The rats were weighed at the beginning of the study, and subsequently every 3 days till the end of the experiment. The kidneys and livers were also weighed after euthanizing the animals on day 7 of intoxication with ochratoxin A. The percentage weight gain or losses were calculated.

**Production of ochratoxin A for animal treatment**

Production of ochratoxin A for animal treatment was carried out using *Aspergillus ochraceus* isolated from cocoa bean seeds. Production was done using the modified methods of Wangikar, *et al.* [21] and Stoev [22]. *A. ochraceus* were grown on Cypeck Yeast Agar (CYA) and after 7 day's growth at 25°C. One millilitre of the conidial suspension was inoculated into a mixture of 100g shredded rice and maize (60:40) in 1000 mL conical flask and moistened to 30% (v/w) with distilled water then incubated for 14 days at 25°C. After incubating, the spores were killed by autoclaving for 1h at 121°C. Extraction of ochratoxin A was done from the solid matrix using the procedure of Association of Analytical Chemist (AOAC) [23]. The matrix was homogenized in sodium bicarbonate solution (3%), centrifuged at 10,000g for 20 min and filtered through No 1 filter paper, after which the filtrate was extracted in chloroform and 0.1 M phosphoric acid for 20 minutes, then the solvent was evaporated and residue dissolved in chloroform again. Ochratoxin A was eluted with methanol and analyzed by TLC and HPLC. The crude product was further purified using silica gel thin-layer chromatography in ethyl acetate-methanol-acetic acid (94.5:5:0.5). Ochratoxin A was crystallized from benzene and found to be 96.7% pure and identical with standard ochratoxin A.

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

Ten milligram per milliliter (10 mg/mL) extract was prepared by adding 2000 mg extract in 200 mL water. One millilitre extract solution contains 10 mg extract translating into 300/1000 x weight of animal. Extract administration was done every three days at 7:00 am in the morning. Oral administration with the aid of an incubator was adopted to force feed (gavage). Ochratoxin A was dissolved in sterile Dimethyl sulphoxide (DMSO) and volume and dose adjusted to animal weight. Dosages were administered intraperitoneally per animal once at different concentrations of OTA. The weight of the animals were monitored and recorded.

**Serum collection in treated albino rats**

At day seven, animals were weighed and subsequently euthanized. Blood specimens were collected from the vein in the tail of animals in each group and pooled together into plain tubes. The animals were then sacrificed by cervical dislocation.

**Biochemical parameters in albino rat blood specimens**

The blood specimens were individually centrifuged at 1085 x g for 15 minutes at 4°C. The specimens were analyzed using kit produced by RANDOX group, USA.

**Urea test**

To 10 µL of serum specimen was added 100 µL of urease reagent I, followed by 2.5 mL each of urease reagents II and III then incubated for 25 minutes at 37°C and read at 546 nm.

**Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) tests**

To 100 µL of serum specimen in a test tube was added 500 µL of AST or ALT reagent I and incubated for 30 minutes at 37°C in a water bath. Thereafter, 5 mL of NaOH was added and read at 546 nm.

**Alkaline phosphatase test**

To two test tubes containing serum was added 1 mL of distilled water followed by, one drop of alkaline phosphatase substrate and incubated for 5 minutes at 37°C. Five milliliter of color developer was added and incubated at 37°C for 5 minutes again and read.

### Total protein test

To 40  $\mu$ L of serum sample was added 20 mL of protein reagent, incubated for 30 minutes at 37°C then read at 546 nm.

### Albumin test

In a test tube, 3 mL of albumin reagent was added to 10  $\mu$ L of serum sample, incubated for 5 minutes at 37°C then read at 630 nm.

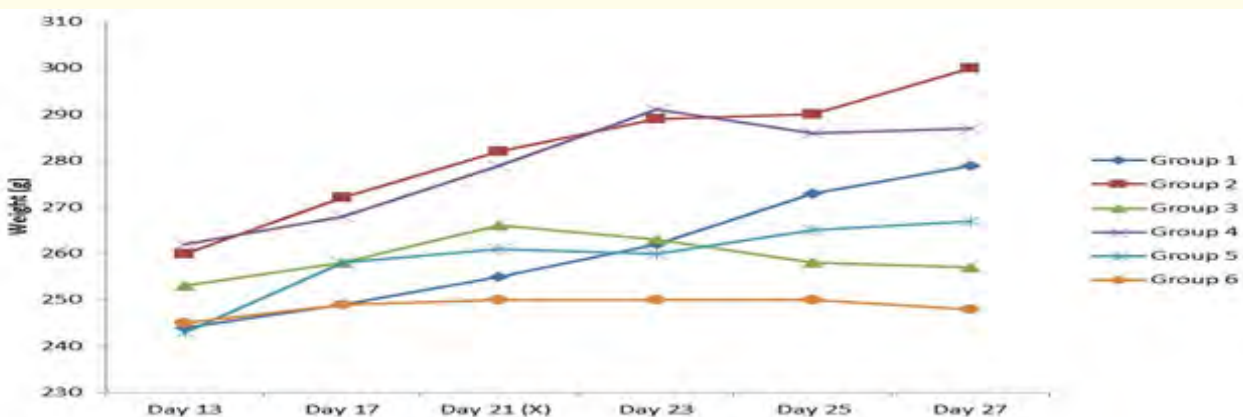
### Statistical analysis

Tukey HSD All-Pairwise Comparisons Tests at 5% was used to compare the means. Analysis of Variance was obtained and the means were separated using Tukey's test at  $p \leq 0.05$ .

## Results and Discussion

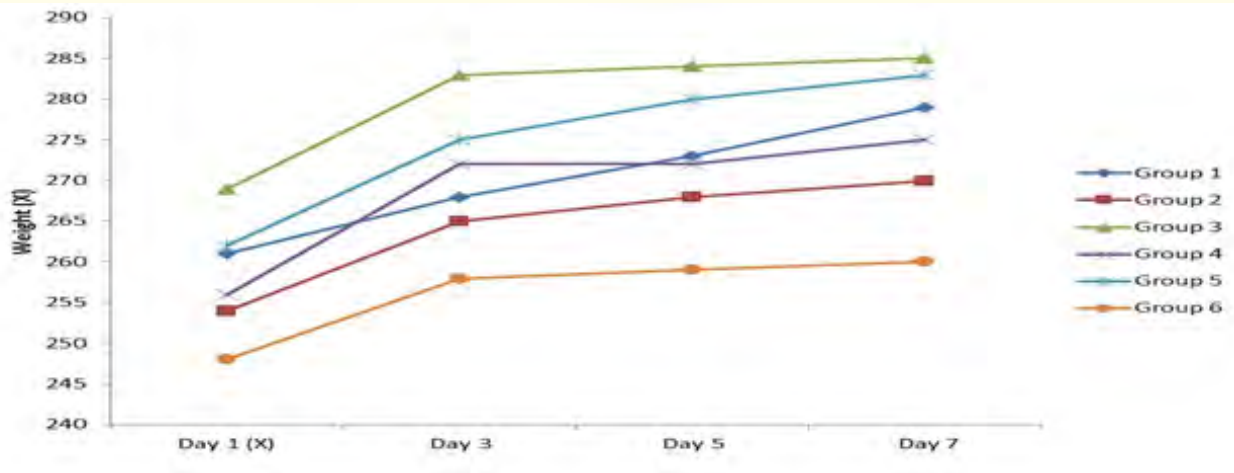
### Effect of ochratoxin A on animal weight

Animal weight in the negative control group increased till the animals were euthanized (Figure 1). Animals administered 300 mg *L. alba* only presented similar observation. Animals in Group 3 and Group 6 obtained increases in weight till the 21<sup>st</sup> day when ochratoxin A was introduced into them; 48h after, weight reduction were observed. Weight decrease was observed in animals of Groups 4 and 5 after OTA administration. In the post-treated animal set, increasing weight trend were noticed among the animals from group to group (Figure 2). Animals that were administered 10% DMSO obtained steady increase in weight till the animals were sacrificed. A sharp increase was observed 48h after the animals were intoxicated with ochratoxin A.



**Figure 1:** Body weight of albino rats pre-treated with *Lippia alba* extracts.

**Key:** X – means day OTA was administered; Group 1: Negative Control – DMSO only; Group 2: Positive Control – 300 mg *L. alba* per kg body weight; Group 3: 2 mg ochratoxin A per 250 mg bw of animals only; Group 4: 1 mg ochratoxin A per 250 mg bw of animals only; Group 5: 2 mg OTA/250 g bw + 300 mg *L. alba* per kg body weight; Group 6: 1 mg OTA/250 g bw + 300 mg *L. alba* per kg body weight.



**Figure 2:** Body weight of albino rats post-treated with *L. alba* crude extracts.

**Key:** X – means day OTA was administered; Group 1: Negative Control – DMSO only; Group 2: Positive Control –300 mg *L. alba* per kg body weight; Group 3: 2 mg ochratoxin A per 250 mg bw of animals only; Group 4: 1 mg ochratoxin A per 250 mg bw of animals only; Group 5: 2 mg OTA/250 g bw + 300 mg *L. alba* per kg body weight; Group 6: 1 mg OTA/250 g bw + 300 mg *L. alba* per kg body weight.

**Organ weight to body weight ratio in *Lippia alba* treated animals**

The ratio of organ weight to body weight of animals (Table 1) showed that the liver to body weight, and kidney to body weight values in group 1 and group 2 were significantly different at  $p < 0.05$  in both animal sets. In *L. alba* pre-treated animals, liver to 100 g body weight for each treatment showed differences which were significant with the least ratio being those in group 2 animals ( $2.51 \pm 0.14$  liver/100 g bw), which steadily increased to  $2.66 \pm 0.09$  liver/100 g bw as seen in group 5 animals. Group 2 animals of the same set (Set 1) gave the lowest ratio in the kidney to 1 kg body weight category when compared with other groups within the set. Group 4 animals reduced to  $4.55 \pm 0.07$  kidney/1 kg bw when compared to the negative control, though it obtained 14 % increment when compared with Group 6 animals of the same set 1. Animals post-treated with the extract obtained values which were significantly different for the different treatment tested, both for liver to 100 g body weight and kidney to 1 kg body weight. Animals in Group 1 and Group 5 obtained values ( $2.29 \pm 0.16$  and  $3.01 \pm 0.02$  liver to 100 g bw respectively). At the concentration of 300 mg/kg bw *L. alba* tested, positive effects against ochratoxin A at 2 mg and 1 mg OTA/250 g bw (Group 5 and 6) were recorded. Ratio between organs and body in the set were significantly different at  $p < 0.05$ .

Treatments	Set 1: <i>L. alba</i> Pre-treated		Set 2: <i>L. alba</i> Post-treated	
	Liver to 100 g body weight	Kidney to 1 kg bw	Liver to 100 g bw	Kidney to 1 kg bw
Gp 1: Negative Control (DMSO only)	$2.51 \pm 0.14^a$	$4.22 \pm 0.01^a$	$2.92 \pm 0.16^b$	$5.06 \pm 0.04^a$
Gp 2: Positive Control (300 mg <i>Lippia alba</i> / kgbw)	$2.78 \pm 0.10^d$	$4.87 \pm 0.16^d$	$2.99 \pm 0.08^c$	$5.70 \pm 0.06^c$
Gp 3: 2 mg OTA/ 250 g bw	$2.93 \pm 0.10^e$	$5.16 \pm 0.13^e$	$3.09 \pm 0.06^d$	$6.88 \pm 0.15^d$
Gp 4: 1 mg OTA/ 250 g bw	$2.75 \pm 0.02^d$	$4.55 \pm 0.07^b$	$3.26 \pm 0.12^e$	$6.67 \pm 0.25^f$
Gp 5: 2 mg OTA/ 250 g bw + 300mg/kg bw <i>L alba</i>	$2.66 \pm 0.09^c$	$4.67 \pm 0.01^c$	$3.01 \pm 0.02^c$	$6.39 \pm 0.30^e$
Gp 6: 1 mg OTA/ 250 g bw + 300mg/kg bw <i>L alba</i>	$2.60 \pm 0.06^b$	$5.20 \pm 0.01^e$	$2.71 \pm 0.06^a$	$5.50 \pm 0.30^b$

**Table 1:** Organ weight to body weight ratio in albino rats pre- and post-administered *L. alba*.

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

**Effects of *Lippia alba* treatments on serum biochemical parameters in ochratoxin A administered rats**

The urea levels in the control animals in all the two sets were within the normal range ( $19.68 \pm 0.7$  mg/dL) as shown in table 2. All the treatments were within the same range except the group 6 with the value  $5.30 \pm 1.6$  mg/dL. Post-treated animals administered OTA at the two concentrations obtained lower urea levels of  $5.5 \pm 0.2$  mg/dL (group 3) and  $4.5 \pm 0.6$  mg/dL (group 4). Improvements were recorded when the animals were treated with *L. alba* extract to obtain  $6.95 \pm 0.3$  mg/dL for 2 mg/250g bw ochratoxin A administered group and  $11.45 \pm 2.3$  mg/dL for the 1 mg/250g ochratoxin A administered animal group. The blood urea content ranged between  $11.0 \pm 2.3$  mg/dL and  $19.13 \pm 1.4$  mg/dL. The results were significantly different from control at  $p < 0.05$ . Total protein in animals belonging to the post-treated set obtained slightly lower values when compared with animals in set 1. Animals administered DMSO only obtained for the Set 1:  $74.0 \pm 1.2$  g/L, while those in Set 2 obtained  $52.5 \pm 2.6$  g/L (Table 2). Treatment of animals with 1 mg/250 g body weight ochratoxin A and 300 mg/kg body weight crude extract of *L. alba* did not substantially affect the value of the total protein for the pre-treated set with  $81.5 \pm 2.0$  g/L, while the same treatment in the post-treated set recorded  $46.0 \pm 2.3$  g/L. Total protein content of the serum specimens were not significantly different from each other at  $p < 0.05$  for animals in Set 1, unlike those in Set 2 whose values were significantly different from the control group.

Group	Treatment	Set 1: <i>L. alba</i> pre-treated	Set 2: <i>L. alba</i> post-treated	Set 1: <i>L. alba</i> pre-treated (g/L)	Set 2: <i>L. alba</i> post-treated
		Urea (mg/dL)		Total protein (g/L)	
1	DMSO only	$19.68 \pm 0.7^c$	$16.20 \pm 0.6^c$	$74.0 \pm 1.2^{ab}$	$52.5 \pm 2.6^{ab}$
2	Extract only	$19.70 \pm 4.2^c$	$15.17 \pm 4.5^{bc}$	$76.2 \pm 4.2^{ab}$	$49.5 \pm 2.6^{ab}$
3	2 mg OTA/250 g bw	$16.20 \pm 0.4^{bc}$	$5.50 \pm 0.2^{ab}$	$80.5 \pm 1.4^{ab}$	$77.0 \pm 1.7^d$
4	1 mg OTA/250 g bw	$14.18 \pm 1.7^{bc}$	$4.50 \pm 0.6^a$	$73.0 \pm 2.3^a$	$56.7 \pm 0.9^{bc}$
5	2 mg OTA/250 g bw + Extract	$12.80 \pm 1.4^b$	$6.95 \pm 0.3^{abc}$	$78.1 \pm 1.7^{ab}$	$64.5 \pm 1.4^c$
6	1 mg OTA/250 g bw + Extract	$5.30 \pm 1.6^a$	$11.45 \pm 2.3^{abc}$	$81.5 \pm 2.0^b$	$46.0 \pm 2.3^a$

**Table 2:** *Lippia alba* treatment effects on urea and protein levels in albino rats intoxicated with ochratoxin A.

Values are means  $\pm$  Std error of mean;  $n = 10$ .  $a1 =$  Means with different letters are significantly different at  $p = 0.05$  (Tukey HSD all-pairwise comparisons test).

Alkaline phosphatase in the pre-treated set (Table 3) had the highest value in animals of group 5 ( $122.3 \pm 2.6$  U/L). Animals in group 2 of the same set, obtained the lowest alkaline phosphatase value of  $35.2 \pm 2.7$  U/L. In the post-treated; animals administered ochratoxin A alone obtained values as high as  $262.0 \pm 7.6$  U/L, while the lowest value of  $65.7 \pm 0.9$  U/L was obtained from animals given DMSO only in Set 2. The data obtained for each set were significantly different from control at  $p < 0.05$ . The highest serum globulin level was obtained in animals in group 2 at  $56.40 \pm 2.7$  g/L, while the lowest value of  $36.30 \pm 5.6$  g/L was obtained in group 5 (Table 3). Animals in group 3 post-treated set obtained  $63.00 \pm 2.0$  g/L as the highest level in the set, while group 5 animals had value of  $48.50 \pm 0.9$  g/L. Values obtained as a result of the treatments administered were significantly different along the column at  $p < 0.05$ .

Group	Treatment	Set 1: <i>L. alba</i> pre-treated	Set 2: <i>L. alba</i> post-treated	Set 1: <i>L. alba</i> pre-treated	Set 2: <i>L. alba</i> post-treated
		Alkaline phosphatase (U/L)		Globulin (g/L)	
1	DMSO only	$65.7 \pm 0.9^b$	$111.3 \pm 3.9^b$	$44.30 \pm 1.0^{ab}$	$37.33 \pm 2.0^b$
2	Extract only	$94.8 \pm 6.7^c$	$128.7 \pm 2.5^b$	$56.40 \pm 2.7^b$	$33.00 \pm 0.6^b$
3	2 mg OTA/250 g bw	$35.2 \pm 2.7^a$	$262.0 \pm 7.6^e$	$45.67 \pm 0.4^{ab}$	$63.00 \pm 2.0^d$
4	1 mg OTA/250 g bw	$68.5 \pm 2.7^b$	$188.0 \pm 6.9^c$	$46.20 \pm 1.5^{ab}$	$28.07 \pm 0.6^a$
5	2 mg OTA/250 g bw + Extract	$122.3 \pm 2.6^d$	$238.0 \pm 3.5^d$	$36.30 \pm 5.6^a$	$48.50 \pm 0.9^c$
6	1 mg OTA/250 g bw + Extract	$88.0 \pm 0.6^c$	$86.9 \pm 1.3^a$	$46.42 \pm 1.4^b$	$31.30 \pm 0.9^{ab}$

**Table 3:** *Lippia alba* treatment effects on alkaline phosphatase and globulin levels in albino rats intoxicated with ochratoxin A.

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

Animals in group 1 of Set 1 and Set 2 were within the normal range ( $26.10 \pm 2.0$  U/L and  $25.23 \pm 1.4$  U/L) (Table 4). The groups recorded values higher than the normal for rat (17.5 – 30.2 U/L). In the post-treated set, group 3 obtained  $68.57 \pm 2.9$  U/L, while group 4 recorded  $63.17 \pm 0.3$  U/L. The values when the animals administered the toxin were treated with 300 mg/kg body weight crude extract of *Lippia alba* were  $62.30 \pm 4.2$  U/L (Set 1) and  $59.17 \pm 2.5$  U/L. In the pre-treated set, animals in group 3 and those in group 6 obtained values of  $58.20 \pm 7.3$  U/L and  $63.50 \pm 0.3$  U/L respectively. Alanine aminotransferase levels in the treatments were higher and values obtained based on the different treatment administered were significantly different from the control at  $p < 0.05$ . Aspartate aminotransferase levels as shown in table 4 for the pre-treated and post-treated sets were high. The negative control animals administered DMSO recorded  $73.36 \pm 1.2$  U/L (pre-treated) and  $67.00 \pm 3.1$  U/L (post-treated), which were in the normal ranges for albino rats (45.7 – 80.8 U/L). Group 4 animals in the pre-treated and post-treated sets obtained the highest values of  $229.63 \pm 1.5$  U/L and  $198.00 \pm 2.0$  U/L respectively, while group 6 had  $157.74 \pm 0.4$  U/L (pre-treated) value and  $222.31 \pm 0.9$  U/L (post-treated). In the *Lippia alba* post-treated set, group 6 animals recorded  $222.31 \pm 0.9$  U/L. Serum aspartate aminotransferase had the highest level for animals in Group 3 ( $109.52 \pm 2.8$  U/L) and lowest for the group of animals administered DMSO only at  $71.64 \pm 0.3$  U/L in set 3. Administered treatment yielded values across the different groups that were significantly different from control at  $p < 0.05$ .

Group	Treatment	Set 1: <i>L. alba</i>	Set 2: <i>L. alba</i>	Set 1: <i>L. alba</i>	Set 2: <i>L. alba</i>
		pre-treated	post-treated	pre-treated	post-treated
		Alanine aminotransferase (U/L)		Aspartate aminotransferase (U/L)	
1	DMSO only	$26.10 \pm 2.0^a$	$25.23 \pm 1.4^a$	$73.36 \pm 1.2^a$	$67.00 \pm 3.1^a$
2	Extract only	$30.96 \pm 1.3^a$	$30.97 \pm 1.0^a$	$79.78 \pm 2.8^a$	$82.67 \pm 5.8^a$
3	2 mg OTA/250 g bw	$62.93 \pm 1.2^b$	$68.57 \pm 2.9^c$	$205.27 \pm 1.3^d$	$188.30 \pm 0.9^b$
4	1 mg OTA/250 g bw	$66.60 \pm 1.2^b$	$63.17 \pm 2.2^{bc}$	$229.63 \pm 1.5^e$	$198.00 \pm 2.0^{bc}$
5	2 mg OTA/250 g bw + Extract	$58.20 \pm 7.3^b$	$63.50 \pm 0.3^{bc}$	$187.90 \pm 4.8^c$	$207.10 \pm 5.5^{cd}$
6	1 mg OTA/250 g bw + Extract	$62.30 \pm 4.2^b$	$59.17 \pm 2.5^b$	$157.74 \pm 0.4^b$	$222.31 \pm 0.9^d$

**Table 4:** *Lippia alba* treatment effects on alanine and aspartate aminotransferase contents in albino rats intoxicated with ochratoxin A.

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

## Discussion

The increase in weight obtained by the animals administered *Lippia alba* only (Group 2) in Set 1 and 2 showed that the animals did not abstain from food in the course of the experimental study. The result presented by animals administered ochratoxin A alone collaborated each other in the *Lippia* treated sets. Bucci [24] attributed organ and body weight changes to possible withdrawal from food. Organ weight to body weight analysis in laboratory animals is an important index for determining toxic effects of chemicals and it is predictive in nature [25]. The increase or decrease obtained in the course of the animal studies could be the result of varying number of factors that significantly impacted the body or organ weights. For the toxicity study, the 7 day duration for monitoring ochratoxicity in the animal model agreed with the protocol described by Sellers., *et al* [26]. The authors explained that within a period of 7 days, liver weight increases were noticed in toxicology studies using potent hepatic enzyme inducing chemical. In the present study, liver weight to body weight ratios were within the normal range for all treatments administered which might suggest that enzyme induction is absent or still minimal as explained by Seller., *et al* [26].

Organ weight to body weight ratio is an accepted index for determining test associated toxicities in liver and kidney as it is a predictive indicator of the effect of an experimental compound. The index shows significant differences in organ weight between treated and un-treated control animals [25,26]. The Society of Toxicologic Pathology (STP) recommended that organ weight may be included in general toxicity studies lasting from 7 days to 1 year. STP further explained that organ weight to body weight ratios is helpful for clarifying treatment-related organ weight changes, while normal organ weight to body weight help eliminate variations due to body weight differences [26].

Higher organ weight to body weight ratios in animals belonging to group 2, administered *Lippia alba* only might be as a result of continued feeding as the animals did not at any point of time abstain from feeding, thus resultant weight increases were recorded in the group. Elevated levels in the ratio of organ weight to body weight in animal models may be adduced to renal or chronic nephropathy and hepatic toxicities associated with kidney and liver respectively as explained by Greaves [27]. The administration of 300 mg/kg bw *L. alba* resulted in lowering of the elevated ratios as seen in animals of groups 5 and 6. In summary, the extract showed capacity to ameliorate which helped restore the body and organ weights.

Castonguay, *et al.* [28] in their report explained that rats are attracted to palatable food thus, increased feeding saw significant increases in weight ratio of animals in group 2 fed mainly *L. alba* and groups 5 and 6 treated with the extract. The authors further added that weight ratio increases resulted from increased food consumption which led to changes in body composition and subsequently increasing residual body mass.

Animals administered with *Lippia alba* all had their blood urea levels within the normal range of 15 - 21 mg/dL. Higher than normal urea values suggests severe liver disease or inappropriate antidiuretic hormone function, while reduced urea levels could be attributed to reduced production of urea or subsequent decrease in ammonia removal. Healing activities was evident in the post-treated set 2 as seen in groups 5 and 6. Animals intoxicated with ochratoxin A alone (Groups 3 and 4) obtained values which were lower than the normal urea values suggestive of severe liver disease or inappropriate antidiuretic hormone function. The reduced urea levels in ochratoxin A administered animals alone and the control group treated to DMSO alone in the *L. alba* post-treated set might result from reduced production of urea and a subsequent decrease in the removal of ammonia. Improvement recorded after treatment with *Lippia alba* extract showed the plant confers healing capabilities on the animal models. In their study, Chies, *et al.* [29] reported the presence of harmonia – an antioxidant in *L. alba* containing Superoxide Dismutase (SOD) which catalyzes dismutation of superoxide ion to oxygen and hydrogen peroxide and catalase (CAT) which has the potential to degrade hydrogen peroxide to harmless water and molecular oxygen. The phenomenon maintains the internal integrity of the cell by avoiding or decreasing oxidative stress resulting from ochratoxin A intoxication.

The total protein contents in the two sets were not affected nor altered by the different extract treatment administered. Concentrations of the extracts and that of ochratoxin A did not alter the total protein values in the animal models though mild variations were obtained. Mild increases in total protein content obtained especially in the pre-treated animal set could be attributed to the preventive effect the plant *Lippia alba* confers. The results of the study do not tally with the presentation made by Benjamin [30] that ochratoxin A increases total protein content in animal models though his experiment was conducted using different ochratoxin A concentrations.

The globulin contents in the animal models intoxicated with ochratoxin A alone at the two different concentrations tested were higher when compared with the control groups among the sets. Increases recorded could be compensatory to protect the integrity of the cell components. Albumin level in the serum is a marker of liver function as it is affected by hepatic protein synthesis [31]. Ochratoxin A binds to free albumin in the serum thus reducing albumin level in the serum. The mild increase in albumin levels (result not shown) recorded across the post-treated set intoxicated with ochratoxin A then treated with *L. alba* were likely as a result of breakage of OTA-DNA adducts or synthesis of albumin to make up for reduction recorded as a result of adduct formation by the extract. Ochratoxin A inadvertently prevented protein synthesis thus causing a possible hepatic dysfunction from excess secretion by the kidney. The albumin content in the different set of treatment was not elevated contrary to report given by Benjamin [30] that OTA induces increases in albumin content. Animals on treatment of *L. alba* alone in the post-treated set reportedly were within normal values (35 - 50 g/L). Low albumin content could also be attributed to intestinal malabsorption, wasting, malnutrition, and possible excessive excretion by the kidneys [32].

Alkaline phosphatase is a marker for hepatic cholestasis. Ochratoxin A intoxicated groups obtained reduced alkaline phosphatase levels in the pre-treated set when the toxin were administered alone at 2 mg/250 g bw concentration, which contrasts result for animals in the same group in the post-treated set ( $104 \pm 12.70$  U/L) which was within the normal range. The reason adduced could be environmental. Increased production of the enzyme signals necrosis within liver tissues. Lowered levels of alkaline phosphatase enzyme recorded in



group 3 of the pre-treated set might have been due to reduced production of the enzyme or its leakage triggered by response to intrusion by an external agent (ochratoxin A) as explained by Wronslewski and Due [33]. Elevated levels of the enzyme in the post-treated set showed a dysfunction of the liver leading to over-production of the enzyme to compensate for protein synthesis, or that mop-up activities within the liver cells had stopped. Reductions recorded after treating with *L. alba* showed that the plant leaves harbours ameliorative active ingredients which confers hepatoprotective cover for the liver and the animals. The study agrees with report of Scola, *et al.* [34] that *L. alba* protects hepatic organs.

Animals in the different groups obtained values that double the normal range 17.5 - 30.2 U/L for ALT, and 45.7 - 80.8 U/L for AST except animals in the control and *L. alba* administered groups alone (Group 1 and 2 respectively). The result showed that the extracts did not alter cell membrane through leaching nor cause the production of free radicals into the cell that could trigger oxidative damage and disrupt protein synthesis. Ochratoxin A substantially increased the values of the transaminase enzymes by leaching into the serum as a result of cellular leakage. Values of AST and ALT in the post-treated set compared to the pre-treated set were higher which were in agreement with the report of Morsy, *et al.* [35] that ochratoxin A induces production of the enzymes and in the process cause reactive oxygen species (ROS) production.

Increase recorded against the ALT and AST levels across the groups showed further, that ochratoxin A is a risk factor for hepatic injury of the liver. The study agrees with the report of Green and Flamm [36] that increased ochratoxin A concentration administration would result to higher levels of the markers in serum where they have been leached to. Another reason adjudged for a possible increment is the use of plant extracts for treatment. The use of plant extract of *Lippia alba* in treatment alone showed mild increases in AST and ALT levels when compared with the controls. Green and Flemm [36] explained that medication, herbal supplement, and drug use might lead to elevated transaminase levels. Kim, *et al.* [37] suggested that the mild increase in ALT value recorded though minor is a predictor of liver disease or injury. *Lippia alba* extract did not significantly reduce the AST levels in the post-treated animal set. The reason could be that, excess OTA-DNA adducts resulting from necrosis had been formed thus making scavenging of free radicals difficult or that the concentration of the extract is low or that more time is needed for the extract to mop-up the enzyme in the serum. Manian, *et al.* [38] explained that the plant extract contain tannins which are high molecular weight active ingredients with capabilities to attract free ochratoxin A radicals. The phenomenon is likely responsible for reduced AST levels obtained in models pre-treated with the extract then later intoxicated with OTA at different concentrations.

### Conclusion

Enzyme assays in serum are known markers for protective, healing, anti-inflammatory, ameliorative, and modulating responses for detoxification activities of plant extracts. Gagliano, *et al.* [39] reported that kidney is a site of intense oxidative processes, and it is vulnerable to damages caused by free radicals. The study presented results on the capabilities of *Lippia alba* to attenuate the supposedly renal injury induced by ochratoxin A through the antioxidants present in the plants. *Lippia alba* from the foregoing have the capacity to heal and restore to normalcy enzymes in the serum, liver and kidney produced in response to foreign substances (ochratoxin A) introduction into the body.

### Conflict of Interest

There are no conflicting interest.

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