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Toxicological Study of Extracts of *Lippia alba* and *Ganoderma lucidum*

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Abstract

The toxicological study is an important phase in drug formulation and developmental process which necessitates the determination of the safe concentrations at which extracts of *Lippia alba* and *Ganoderma lucidum* present no toxic side-effect and symptoms in albino rats. Fifty albino rats weighing 150g-170g divided into *L. alba* group and *G. lucidum* group were administered with 100 to 800 mg/kg body weight (BW) concentrations of the extracts, respectively, and observed for 14 days. The animals were subsequently euthanized and blood samples collected were tested for hematological and biochemical parameters, while the liver, kidney, and heart sections were excised for histopathological examination. Results showed that the animals' behavior was not at variance from the control, except in group 2 (100 mg extract/kg BW) of both *L. alba* and *G. lucidum* and group 4 (400 mg extract/kg BW) of *G. lucidum*, body weight was lower and liver weight per 100g BW was higher compared to other treatment groups. Leukocytes, hemoglobin, platelets, erythrocytes and hematocrit values were not statistically different in both *L. alba* and *G. lucidum* groups compared to control. Similarly, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, globulin, albumin and total protein content were also not statistically different in both *L. alba* and *G. lucidum* groups compared to control. Kidney, liver, and heart sections presented no visible damage due to the administered treatments. In conclusion, the extracts of the plant and mushroom at the concentrations between 100 and 800 mg/kg BW did not lead to any observable toxic effects in the animal models.

Introduction

Reports of the effectiveness of herbal and mushroom preparations abound and lately increased use of herbal and mushroom products have been observed because of the belief that they are natural and present no toxic effects unlike the orthodox medicine counterpart [1]. The use of different plants and products of plant origin has increased in the treatment of ailments and infections all over the world, and it is becoming increasingly acceptable [2-3]. The use of plant materials is further encouraged by the cheap nature and ease of accessibility of the plants. Toxicological studies are necessitated by the challenges raised by drug resistance, drug interactions and side-effects, dose-limiting toxicities, and stages of drug formulation [4]. The challenge of food scarcity, re-emerging diseases and infections, global warming, coupled with a dearth of relevant information on the safety and effects of herbal products and preparation make toxicological studies important. Plant and mushroom parts contain an array of compounds and metabolites which at particular concentrations could pose toxic effects to human health by impairing organ function and cellular dysfunction [5].

Ganoderma lucidum can be used as a fungal portion to aid in balancing the adrenal system and improve the body's response to daily stress. Its mechanisms of action are diverse, but are usually localized around moderating the immune system, increasing the amount of active cells and the potential for their effects. It has anti-oxidative effects, and can act on a few other systems such as aldose reductase, which can help with diabetic symptoms and 5-alpha-reductase (which help with prostate cancer risks) [6]. Due to these effects paired with the modulation of the immune system, *G. lucidum* shows promise of being *therapeutic* for insulin resistance, prostate cancer risk, and a variety of conditions that correlate with the metabolic process. It is also well known and touted for its anti-cancer effects [7].

Lippia alba infusions are traditionally used as sedatives, and to treat digestive upset, diarrhea, and relief of stomach pains [8, 9]. It has been found to be cytotoxic, antifungal, antibacterial, antiviral, sedative, febrifuge, carminative, antispasmodic and anti-inflammatory [10, 11]. The plant also contains geranial, neral, geraniol, trans-β-caryophyllene and plant oils that contain oxygenated monoterpenes, limonene, carvone, bicyclosesquiphellandrene which confers it with high antifungal activity [12]. Considering the medicinal importance conferred by *L. alba* and *G. lucidum*, the study aims to determine the concentrations at which extracts of the plant and mushroom become toxic and unsafe for use. The aim would be achieved by determining the effect of different doses of the plant and mushroom preparations on the biochemical and hematological parameters in the liver and blood of albino rats.

Materials and Methods

Collection and identification of materials

Lippia alba leaves and fruiting bodies of *Ganoderma lucidum* were obtained from Akungba-Akoko and Iwaro-Oka in Ondo State, Nigeria, respectively. Those were identified and authenticated by Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko. After identification, the materials were washed and dried under shade for twenty-one (21) days, after which they were separately blended into fine powder, labeled and stored in the refrigerator at -20ºC.

Extraction of plant and mushroom extracts

The modified methods of Orole et al. [13] and Odey et al. [14] were employed in the extraction process as follows: four hundred grams (400 g) each of the *L. alba* leaves and *G. lucidum* mushroom was separately weighed and soaked in 2000 ml of methanol (98%) at a ratio of 1:5 (powder: solvent). The mixtures were kept in airtight containers and left for 48 hours at room temperature. Filtrations to remove residue were done using a double layer muslin cloth, followed by another stage of filtration using Whatman No 1 filter paper (24 cm). The filtrate was then separately concentrated *in vacuo* using a rotary evaporator (Model RE52A, China) to 10% of the original volume at 37 ºC – 40ºC. The final concentrations to dryness were carried out by evaporating to dryness in a water bath at 60ºC. The percentage yield (w/w) from the extraction process was calculated as follows:

$$\text{Yield (\%)} = (W1 \times 100) / W2$$

Where W1 is the weight of the extract after evaporation to dryness with solvent, and W2 is the weight of dried plant powder.

Collection and preparation of albino rat models for acute toxicity study

Acute toxicity tests for *L. alba* and *G. lucidum* were carried out with fifty albino rats weighing between 150 g and 170 g. The animals were kept according to the guide for the care and use of laboratory animals in research and teaching proposed by the National Research Council, USA [15].

Acute toxicity test of plant and mushroom extracts

The modified methods of Oyewo et al. **[**16**]** were adopted. Animals were divided into *L. alba* group and *G. lucidum* group, respectively. Each group was further sub-divided into a group of five animals and treatments were administered as follows:

Group 1: only 10% DMSO for 14 days (Control)
Group 2: 100 mg/kg BW extract for 14 days
Group 3: 200 mg/kg BW extract for 14 days
Group 4: 400 mg/kg BW extract for 14 days
Group 5: 800 mg/kg BW extract for 14 days

Administration of the extract was done once by gavage at 7 AM in the morning after night fasting of the animals. Oral administration with the aid of an incubator was adopted to force feed (gavage). Animals after administration of the single dose of the extract were monitored for changes in behavior, signs of toxicity, and mortality in the first 4 h of administration. Further observation of the rate of urination, food and water intake, the rate of respiration, signs of tremor and convulsions, increase in temperature, constipation, and eye and skin color changes were noted and recorded.

Determination of relative organ weight

The liver, kidney and heart of euthanized albino rats were carefully excised, blotted, and weighed. The relative organ weight of each animal was calculated using the equation:

Relative organ weight = Absolute weight of organ (g) × 100 / BW on the day of sacrifice (g)

Estimation of hematological and biochemical parameters

The animals were euthanized on the 14^{th} day after extract administration using chloroform after overnight fasting that lasted 8 h according to the World Medical Association's Helsinki Declaration which governs the use of laboratory animals [17]. The blood samples were obtained by cardiac puncture into test tubes with and without ethylene diamine tetra acetic acid (EDTA) for hematological and biochemical parameters according to the modified methods of Builder et al. [2]. Blood for biochemical parameters was centrifuged as described in the kit protocol for determining alanine aminotransferase, aspartate aminotransferase enzymes, alkaline phosphatase, urea, globulin, albumin, and total protein content, respectively. Blood specimens collected in EDTA anticoagulant bottles were subsequently used to determine erythrocyte and leukocyte counts, hemoglobin levels, platelets and hematocrit volumes, respectively.

Histopathological examination of organs

Sections of the heart, liver, and kidney were prepared and fixed using the modified methods of Pieme et al. **[**18**]** by cleaning in 10% formaldehyde for 24 h and subsequently dehydrated in alcohol diaphonized in xylol soaked in paraffin. Five-micrometer (5 µm) thick sections were prepared from each organ, stained with hematoxylin and eosin (HE) and observed under the microscope for damages which were graded according to the intensity of destruction, and give a score thus: 0 = no damage; 1 = discrete damage; 2 = moderate damage; 3 = severe damage.

Statistical analysis

The result of blood and organ parameters were expressed as means and standard deviations, while analysis of variance (ANOVA) was obtained using SPSS software, and the means were separated using Bonferroni post-tests at $P \leq 0.05$.

Results

Behavior and body observation of rat after treatment with extracts

The animals administered extracts at different concentrations were observed not to have deviated from the appearance and behavior when compared to the control groups in the two sets used for acute toxicity experimentation. As the experiment proceeded, body temperature was within the same

Table 1 Body and relative liver weight of albino rats treated with concentrations of extracts of *Lippia alba* and *Ganoderma lucidum.*

Groups	*Lippia alba*		*Ganoderma lucidum*	
	Body weight (g)	Liver weight/100g body weight	Body weight (g)	Liver weight/body weight ratio
Group 1	182.3±2.1	2.198±0.6	186.3±1.1	2.151±0.3
Group 2	176.0±1.4[a]	2.272±0.1[a]	177.1±0.8[a]	2.259±0.9[a]
Group 3	180.4±0.9	2.222±0.1	185.2±2.3	2.160±0.6
Group 4	181.5±2.0	2.203±0.5	179.8±0.2[a]	2.225±0.7[a]
Group 5	184.4±1.7	2.174±0.6	181.1±3.2	2.209±0.8

Group 1 = Control, Group 2 = 100 mg extract/kg BW; Group 3 = 200 mg extract/kg BW, Group 4 = 400 mg extract/kg BW; Group 5 = 800 mg extract/kg BW
Values are represented as mean ± standard deviation. "a" represents a significant difference when compared with the control at $p \leq 0.05$ using Bonferroni post-tests.

range compared to the control group, and urination and feeding were normal, while changes in skin and eye colors were not found. The animals did not show any symptoms of weakness, sedation, drowsiness, and none of the animals died until they were euthanized (data not shown).

Body weight and relative liver weight ratio

Table 1 presents the body weights of animals at day 14th and the liver weight to 100 g body weight of animals administered with *L. alba* and *G. lucidum* extracts. The results of animal body weights for *L. alba* group showed that the body weight in group 2 was the lowest (176 g) and significantly lower than control, while animals in group 5 administered 800 mg extract per kg BW showed the highest body weight but not significantly different than control. Animal administered *G. lucidum* group obtained values ranging from 177.1 g for group 2 animals (lowest) to 186.3 g for group 1 (highest). The values obtained for body weight were not significantly different compared to control at $P \leq 0.05$, except for group 2 and group 4. The liver weight to body weight ratios between the two animal groups did not show any deviation from the control group, except group 2 in *L. alba* group and group 2 and group 4 in *G. lucidum* group at $P \leq 0.05$.

Effects of plant extracts on blood parameters

Treatment of albino rats with different concentrations of *L. alba* and G. *lucidum* extracts, respectively, when compared with the control groups showed that the values were within the reference levels as shown in Table 2. In the *L. alba* treated set, erythrocytes (RBC) had the least value in the group 2 administered with 100 mg extract (6.38×10^3/µl), leukocyte (WBC) in group 1 administered with 10% DMSO, platelets in group 5 administered with 800 mg extract (447.05×10^3/µl), and hemoglobin and hematocrit in group 3 administered with 200 mg/kg BW extract of *L. alba* (13.33 g/dl and 38.68%, respectively). In the set administered with *G. lucidum* extract, erythrocyte obtained the lowest value in group 4 administered with 400 mg/kg BW extract (6.53×10^3/µl), leukocyte in group 1 administered with 10% DMSO (9.44×10^3/µl), platelets in group 4 administered with 400 mg/kg BW extract (512.22×10^3/µl), hemoglobin in group 3 administered with 200 mg/kg BW extract (13.98 g/dl) and hematocrit in group 5 administered with 800 mg/kg BW extract (35.22%). The different means were not significantly different from the control at $P \leq 0.05$, except for platelet counts when animals were administered with 200 and 800 mg *L. alba* extract per kg BW, and hematocrit percentage in the treatment of 800 mg *G. lucidum* extract per kg BW, respectively.

Effect of plant extracts on biochemical parameters

The effects of *L. alba* and *G. lucidum* extract treatments on blood parameters is reported in Table 3. The results showed that alanine aminotransferase and aspartate aminotransferase activities and albumin and urea content showed nonsignificant differences in all groups of *L. alba* and *G. lucidum* extract treatments at $P \leq 0.05$. The alkaline phosphatase activity was significantly higher in group 5 (800 mg extract/kg BW) of *L. alba* extract treatment compared to control, while other groups showed nonsignificant differences with the control. Total protein content was significantly higher in group 4 (400 mg extract/kg BW) of *L. alba* extract treatment compared to control, while other groups showed nonsignificant differences with the control. Globulin contents of

Table 2 Effect of different concentrations of extracts of *Lippia alba* and *Ganoderma lucidum* on blood parameters of albino rats.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
			***Lippia alba* extract treatment**		
Erythrocyte ($\times10^3$/µl)	7.13±1.7	6.38±0.3	6.90±0.6	7.90±1.2	6.87±1.0
Leukocyte ($\times10^3$/µl)	7.42±1.5	9.04±2.1	9.89±0.7	9.54±0.6	8.88±1.1
Platelets ($\times10^3$/µl)	512.23±30.4	493.77±22.1	461.09±16.2[a]	475.15±6.7	447.05±42.2[a]
Hemoglobin (g/dl)	14.49±0.33	15.12±1.2	13.33±1.0	14.05±2.7	14.89±0.7
Hematocrit (%)	42.89±2.5	39.56±3.0	38.68±2.5	40.34±1.9	38.80±3.6
			***Ganoderma lucidum* extract treatment**		
Erythrocyte ($\times10^3$/µl)	7.35±0.7	6.81±0.4	6.72±1.6	6.53±0.3	6.56±1.3
Leukocyte ($\times10^3$/µl)	7.92±0.7	9.11±1.3	8.35±0.3	9.44±1.0	8.99±0.9
Platelets ($\times10^3$/µl)	538.05±9.5	545.83±22.1	522.31±17.3	512.22±7.5	519.17±30.1
Hemoglobin (g/dl)	14.40±1.1	14.60±0.2	13.98±0.3	14.01±1.4	14.20±1.5
Hematocrit (%)	44.10±6.2	39.66±7.4	42.61±4.1	37.82±3.0	35.22±6.9[a]

Group 1 = Control, Group 2 = 100 mg extract/kg BW; Group 3 = 200 mg extract/kg BW, Group 4 = 400 mg extract/kg BW; Group 5 = 800 mg extract/kg BW
Values are represented as mean ± standard deviation. "A" represents a significant difference when compared with the control at $p\leq0.05$ using Bonferroni post-tests.

Table 3 Effect of different concentrations of extracts of *Lippia alba* and *Ganoderma lucidum* on biochemical parameters of albino rats.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
			***Lippia alba* extract treatment**		
Alanine aminotransferase (U/L)	21.45±1.2	18.88±0.7	18.67±1.3	18.25±1.0	18.98±0.2
Aspartate aminotransferase (U/L)	55.23±1.3	60.46±1.7	60.53±1.3	60.52±1.8	65.31±1.7
Alkaline phosphatase (U/L)	68.83±0.7	71.72±0.6	74.67±0.8	70.90±1.0	77.04±1.4[a]
Total protein (g/L)	61.15±0.8	69.43±1.5	64.42±0.4	79.77±0.9[a]	65.13±1.1
Albumin (g/L)	39.68±4.2	39.30±1.7	41.68±0.9	43.49±1.3	45.25±2.9
Globulin (g/L)	22.89±0.91	31.42±0.5[a]	23.05±1.2	37.65±0.4[a]	20.55±1.1
Urea (mg/dL)	17.62±2.7	17.75±1.6	17.34±0.6	17.03±0.8	16.96±2.3
			***Ganoderma lucidum* extract treatment**		
Alanine aminotransferase (U/L)	21.12±0.9	21.95±0.4	22.12±1.9	19.20±0.7	22.49±0.8
Aspartate aminotransferase (U/L)	58.09±1.4	60.88±1.2	61.79±1.3	54.05±1.0	60.44±1.7
Alkaline phosphatase (U/L)	67.89±1.8	72.20±1.5	74.32±1.3	73.07±1.8	75.44±2.2
Total protein (g/L)	62.06±5.8	72.01±1.4	71.99±8.1	64.08±5.2	68.44±1.8
Albumin (g/L)	39.78±1.7	38.60±5.1	45.20±6.9	40.07±3.0	41.30±4.1
Globulin (g/L)	23.21±0.4	34.31±1.1	29.44±0.7	22.80±1.6	27.07±0.4
Urea (mg/dL)	17.34±0.6	17.30±0.7	16.05±0.8	19.13±0.8	19.33±1.8

Group 1 = Control, Group 2 = 100 mg extract/kg BW; Group 3 = 200 mg extract/kg BW, Group 4 = 400 mg extract/kg BW; Group 5 = 800 mg extract/kg BW
Values are represented as mean ± standard deviation. "A" represents a significant difference when compared with the control at $p\leq0.05$ using Bonferroni post-tests.

group 4 (400 mg extract/kg BW) and group 2 (100 mg extract/kg BW) of *L. alba* extract treatments were significantly higher compared to control, while other groups showed nonsignificant differences with the control. The values obtained for the different treatments were not statistically different from the control at $P\leq0.05$ in animals administered with *G. lucidum* extract.

Effect of extracts on morphology of rat organs

Liver, heart, and kidney of the animals stained with hematoxylin and eosin did not present signs of damage to cells and muscles (data not shown). The control animals were not different from those in the groups administered with *L. alba* and *G. lucidum* extracts. The three organs studied for damages presented no visible damages and were graded zero.

Discussion

Toxicity confers liver injury which is a mixture of different metabolic process dysfunctions. This includes DNA damage cum blocked protein synthesis, increased catabolism involving phenylalanine, leakage of damaged tissues, lipid peroxidation, and mitochondrial respiration interference [19, 20]. The authors reported that increased enzyme levels in the serum are a function of enzyme leakage from the liver cystole, enzyme release by tumor invaded tissues, or the resultant effects of the tumor on the surrounding tissues. Acute toxicity study of *L. alba* and *G. lucidum* was investigated to determine whether the extracts have any adverse effects on liver, kidney and heart of albino rat models. Pre-experimental toxicity testing

helps to determine the "No Observed Adverse Effect Level" (NOAEL), which is needed to initiate the evaluation of a test substance, drug, or toxin. Toxicity tests are imperative to examine specific adverse effects or specific endpoints such as diseases and toxicity in animals. At the extracts' concentrations tested, no weight and behavioral changes were observed in the tested animals, suggesting that the extracts did not produce any pathological damage to animal organs in the course of 14 days.

The results of the study showed that *L. alba* and *G. lucidum* do not produce any symptoms nor signs pointing to intoxication. The animals administered with varying concentrations of extracts of *L. alba* did not produce any noticeable behavioral changes. The study contradicted the findings by Zetola et al. [21] in which ethanolic non-volatile fractions of *L. alba* produced signs and symptoms of sedation and muscle weaknesses in animal models. Animals administered with *L. alba* and *G. lucidum* had their blood urea within the normal range of 15-21 mg/dL. Higher than normal urea values would have suggested severe liver disease or inappropriate antidiuretic hormone function, while reduced urea levels could be attributed to the reduced production of urea or subsequent decrease in ammonia removal. The total protein contents were not altered by the different extract treatments administered, while alkaline phosphatase, a marker for hepatic cholestasis was within the normal range. Although, a slight but significant increase was observed in *L. alba* extract administered animals at the concentration of 800 mg extract/kg BW.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are markers of liver injury [22]. Any small elevation in the level of ALT in the plasma calls for concern [23, 24], as the marker is specific for hepatic injury and is found majorly in the cytosol of the hepatocyte of the liver in high concentration. Injury, stress conditions, and cellular damage all increase ALT value in the serum. Green and Flamm [25] explained that medication, herbal supplement, and drug use may cause elevated transaminase levels. In healthy populations, the acceptable elevated value of ALT should not exceed 2.7%. The study showed that increase in ALT in the pre-treated groups was lower than those in the post-treated categories; reasons adduced might be as a result of the environment or the fact that the residual concentration of plant extract has no healing properties. Kim et al. [26] suggested that the mild increase in ALT value recorded, though minor, is a predictor of liver disease or injury. *G. lucidum* at 100 mg/kg BW by ingestion protects the macrophages from oxidative damage *in vivo* (mice) and prevent morphological changes to the mitochondria and endoplasmic reticulum. The mushroom also rehabilitates damaged mitochondrial membrane in the process [27, 28] and confers a protective effect [29].

The results of acute toxicity study also showed that there was no increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, suggesting that the extract of *L. Alba* at various concentrations tested do not cause hepatotoxic damage to the liver like the essential oil that presented a noticeable increase in ALT levels in albino rats in a previous report [30]. Transaminases are good indices of liver, heart, and kidney damages [4]. Other serum biochemical parameters investigated; urea, total protein, globulin, and albumin levels were all within the normal reference limits. These biochemical indices suggested no damages were done to the liver or the kidney, thus confirming that extracts of the two materials at different concentrations administered were not hepatotoxic nor do they lead to renal dysfunction in the kidney. Blood parameters are predictive of toxicity in animal experiments [31, 32]. Hematological indices presented results that were in consonance with the values obtained for albino rat models in the control groups. The results signal the fact that the administered extracts did not cause lysis as easily noticed in erythrocyte level; anemia, which is suggestive of toxicity of the extract was absent. The results of the blood parameters showed that the plant extracts are safe, and not toxic at the doses administered in a 14-day test. Administration of *L. alba* and *G. lucidum* to animals at concentrations between 100 and 800 mg/kg body weight presented no abnormal

physiology in the kidney, liver, nor the heart. The results showed no dose-dependent histopathological alteration when compared to the organs in the control animal groups, lending support to the safety of the plant extracts when the administration is through the oral route.

Conclusions

The study showed the therapeutic potentials conferred by a mushroom (*Ganoderma lucidum*) and a plant (*Lippia alba*). The extracts of both materials did not show toxic effects at the concentrations studied. The study concluded that at doses between 100 and 800 mg/kg body weight, oral administration of *Lippia alba* and *Ganoderma lucidum* do not cause any toxicities in biochemical, hematological, or histopathological indices in albino rat models, thus ascertaining the safety of the extracts for further experimental study.

Conflict of Interest

The authors have no conflict of interest.

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