EFFECTS OF SUB-ACUTE ADMINISTRATION OF *GANODERMA LUCIDUM* METHANOL EXTRACT ON SOME SERUM BIOCHEMICAL PARAMETERS IN NORMAL WISTAR RATS

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ABSTRACT

Some serum biochemical parameters such as ALP, AST, ALT, Urea, Creatinine and Bilirubin were investigated in normal albino rats following daily oral administration of *Ganoderma lucidum* methanol extract using standard protocols. Doses of 200, 400 and 800 mg/kg were consecutively administered for 21 days, and withdrawal period of 14 days post stoppage of treatment with the extracts was used to monitor post withdrawal effects of treatment. Results from the findings showed dose dependent effect of oral administration of the extract, while at lower doses, there were decreases in serum levels of the biochemical parameters under study, at higher doses there were significant (p<0.05) increases in the serum biochemical parameters under study. Findings from this study reveals the health benefits of extracts from this mushroom at lower doses and its possible toxic effect at high doses following prolonged administration.

KEYWORDS: *Ganoderma lucidum*, methanol extract, biochemical parameters, Wistar rats.
INTRODUCTION
A mushroom consists of the mycelium, which grows inside the soil or wood and the fruiting body seen growing outside. Lower fungi are known to yield important drugs like the penicillin and sulphur amino acids that are beneficial in medicine (Dubey and Masheswari, 2003).

Some of these mushrooms such as wild *G. lucidum* are used as antimicrobials. Examples are anti smallpox mushroom, antibiotic mushroom, cytotoxic mushroom and also libido enhancing mushroom. Proteins from mushrooms are known to enhance cell mediated immune response *in vitro* and *in vivo* (Guo *et al.*, 2003). T-cell immune response characterized by secretion of interferon, cytokine stimulation and macrophage activity are enhanced by mushrooms (Hattori *et al.*, 2011). They also stimulate the growth of immune organs (cytokines) in Newcastle disease infected chickens (Wei *et al.*, 1997).

Compounds such as ganoderic acid, polysaccharides and ganoderin found in *G. lucidum* are very complex with 25 amino acids and equally having multiple mechanisms of actions against microbes (Holliday, 2009). This stimulates growing interest in the use of both edible and medicinal mushroom for food and medicine in Africa and Europe (Pang *et al.*, 2000). The passage of Dietary Supplement Health and Education Acts of 1994 in the United States has placed herbs as “Dietary supplement”. (Stavinoha, 2011).

In China and Japan, the use of *G. lucidum* as a remedy for myriad of diseases such as inflammation, chronic arthritis, and other infections have been known for over 2000 years (Wasser, 2005). It was also mythically used in the Orient as a talisman to protect a person or home against evil (Chang and Buswel, 1999). Extracts from this mushroom are reported to have hepatoprotective effect, and the liver enzymes are used as indicators of their effects. This study is designed to assess the effect of methanol extract of *G. lucidum* on some biochemical parameters in wistar rats.

MATERIALS
Collection and identification of the mushroom
Fresh fruiting part of the wild mushroom (*G. lucidum*) was harvested from Lafia, Nassarawa State in North-central Nigeria during the month of August-September, 2011 (rainy season) and was transported to Maiduguri inside a clean polythene bag. The mushroom was identified and authenticated by an expert mycologist at the Department of Biological Sciences,
University of Maiduguri, Nigeria. It was then air-dried in the laboratory and ground to a fine powder using clean mortar and pestle and stored in an air-tight glass jar at room temperature until required.

**Extraction of the wild G. lucidum**

The dried powder of *Ganoderma* was weighed using a metler balance (Toledo-PB 153, Switzerland), and 1.5 kg of the dried powder was placed in a thimble and was put in a Soxhlet extractor, and extracted exhaustively with 7.5 litres of absolute methanol, extract was evaporated within a period of 24 h, using electric evaporator.

The percentage yield was calculated as follows:

\[
\% \text{ yield} = \frac{W_1 - W_2}{W_1} \times 100
\]

Where \( W_1 = \) initial weight of dried *Ganoderma* powder. \( W_2 = \) final weight (g) of dried *Ganoderma* powder.

**Laboratory Animals**

One hundred albino rats of weights 112 g and 140 g, and aged between three and four weeks of both sexes were randomly allotted into four groups (A, B, C, D,) with twenty five rats in each group. Crude methanol extract of the wild *G. lucidum* was administered orally at varying doses of 200, 400 and 800 mg/kg to three (3) groups A, B and C. While group D was administered with distilled water for the period of three weeks, and the remaining 2 weeks were not treated to observe for reversal in all the treatment groups. Each week of the five weeks (day 7\(^{th}\), 14\(^{th}\), 21\(^{st}\), 28\(^{th}\) and 35\(^{th}\)), five rats from each group were sacrificed by severing the jugular vein (C.I.O.M.S 1985). Thereafter, 1.5 ml of blood collected into bijou bottles from each rat. This blood was divided into two equal parts, one part was allowed to clot to collect serum. The serum was used to analyze for biochemical parameters.

**METHODS**

**Determination of aspartate aminotransferase**

The concentration of aspartate aminotransferase (AST) was determined by methods described by Reitman and Frankel (1957). Two test tubes were set up and labeled A (reagent blank) and B (serum sample). Test tube A (blank) contains 0.5 ml phosphate buffer solution and 0.1 ml distilled water. Test tube B (test sample) contain 0.5 ml phosphate buffer solution and 0.1 ml serum sample (\( R_2 \)) at pH 7.4. To test tube A (blank) and B (test sample) 0.5 ml of phosphate
buffer solution was individually added. In addition 0.1 ml of distilled water was added to test tube A, while to test tube B, 0.1 ml of the serum sample was added thus, making the contents of the two test tubes 0.6 ml. All the mixtures were incubated at 37°C for 30 minutes. Furthermore, 0.5 ml of \( R_2 \), (2,4-dinitrophenylhydrazine) was added to both the test tubes and allowed to stand for 20 minutes at 20-25°C. Finally, 5.0 ml of sodium hydroxide solution was then added to the two test tubes. The absorbance of the solutions were read against the reagent blank at 546 nm after 5 minute.

The result obtained was expressed as microgram per litre (µ/l).

**Determination of alanine aminotransferase**

The concentration of alanine aminotransferase (ALT) was determined *in vitro* by methods described by Reitman and Frankel (1957). One hundred Micromolar of phosphate buffer solution at pH 7.4 (reagent 1) was prepared by dissolving 200 mmol/l of L-alanine and 2.0 mmol/l of \( \alpha \)-oxogutarate in 100 ml of distilled water. Two millimoles in a litre of 2, 4-dinitrophenylhydrazine (reagent 2) was provided by the laboratory and used. Two test tubes, A (Reagent blank) and B (sample test tube) were prepared and phosphate buffer solution (0.5 ml) was added to both the test tubes, and 0.1 ml of distilled water to test tube A. While serum sample was added (0.1 ml) to test tube B, to give a volume of 0.6 ml in each test tube. It was then mixed and incubated at 37°C for 30 minutes. Also 0.5 ml of reagent 2 was added to both test tubes and mixed again and allowed to stand for 20 minutes at 20-25°C. Then, 5.0 ml of sodium hydroxide was added to both test tube A and B and was mixed manually. Absorbance of the sample was read at 546 nm against that of the reagent blank after 5 minutes. Alanine aminotransferase is normally measured by monitoring the pyruvate hydrazone formed with 2, 4- dinitrophenylhydrazine. The result obtained was expressed as microgram per litre (µ/l).

**Determination of serum alkaline phosphatase**

Serum alkaline phosphatase (ALP) was determined *in vitro* by phenolthalein monophosphate method as described by Babson *et al* (1966). Seven millitres of chromogenic substrate (Quimica clinica aplicada S.A Spain) (A), colour developer (250 ml) was prepared by dissolving 2 ml vial in 250 ml distilled water (B) and 5.0 ml of standard (prepared by dissolving 1 ml of alkaline phosphatase in 5 ml distilled water) equivalent to 30 µm/l as (C). Two test tubes were prepared. Reagent blank (a), and sample test tube (b) were prepared. Using a 1.0 ml pipette and then distilled water was added (1.0 ml) into both test tubes (a) and (b), and a drop of the chromogenic substrate was also added. They were mixed and then
incubated at 37°C for 5 minutes. To test tube a, 0.1 ml of the serum sample was added and 0.1 ml of distilled water was added to test tube b. They were both mixed and incubated at 37°C for 20 minutes. Colour developer (5.0 ml) was then added to both the contents of the test tubes and allowed to stand for 1 hour before the absorbance was read at 550 nm wavelengths. Serum alkaline phosphatase concentration in (µ/l) was calculated by dividing the optical density absorbance values obtained in test tube (a) by that obtained from test tube (b) and multiplying by 30 (dilution factor).

Thus,

\[
\text{Alkaline phosphatase} = \frac{\text{Sample O.D}}{\text{Standard O.D}} \times 30 \ (\mu/l)
\]

Where O.D is the measure of the optical density of the test sample and the standard.

**Determination of urea**

Serum urea was determined by using diacetyl monoxime method as described by Afonja (1997). Briefly, 1 ml of each colour reagent and mixed acid reagent were separately added to 20 µl of serum sample and distilled water (blank) in a test tube. The contents of the test tube were mixed thoroughly and were placed in boiling water for 20 minutes at 100°C. Thereafter, the tubes were allowed to cool and the optical density (O.D) of the solutions was read at a wavelength of 520 nm using the blank as control.

The level of urea in the serum sample was calculated using the formula:

\[
\text{Urea conc.} = \frac{\text{Sample O.D}}{\text{Standard O.D}} \times 10 \ (\text{mmol/l})
\]

**Determination of creatinine**

The Jaffe reaction method of Seaton and Ali (1984) was used to determine concentration of creatinine in the serum of treated rats. Briefly, 0.05 ml of the test serum was added to 1.5 ml of distilled water, 0.5 ml of 10 % of sodium tungstate and an equal volume (0.5 ml) of NH₂SO₄. The mixture was centrifuged at 1000 g for 3 minutes. Similarly, 2 ml of distilled water was mixed with 0.5 ml of 10 % sodium tungstate and 0.5 ml of NH₂SO₄. It was vigorously shaken and centrifuged at 1000 g for 3 minutes and served as control. To 1.5 ml of the supernatant of both the test sample and the control, 0.5 ml each of the standard solution, 0.75 N NaOH and of picric acid were added. The mixture was left to stand for 15 minutes, and absorbance read at a wavelength of 520 nm. The concentration of the creatinine in each serum sample was calculated. The result expressed as micromoles per litre (mmol/l)
Determination of total bilirubin

This was determined by calorimetric method of Jendrassik and Grof (1938) using commercial kits (Randox Lab. LTD. Ardmore, U.K). Briefly, 0.1 ml, 0.05 ml and 1.0 ml of the reagent 1, 2 and 3 respectively, were added to 0.2 ml of either serum or standard solution. The mixtures were allowed to stand for 10 minutes at room temperature. Thereafter, 1.0 ml of reagents 4 was added to each solution and allowed to stand for 5-10 minutes at room temperature after which the absorbance of the solutions were read against that of the standard at a wavelength of 578 nm.

The reading was used to calculate serum total bilirubin concentration using the formula:

\[
\text{Total bilirubin conc.} = \frac{\text{Sample O.D}}{\text{Standard O.D}} \times 10.8 \text{ (mg/dl)}
\]

Where 10.8 is a multiplication factor.

RESULTS

Effects of sub acute administration of crude extract of wild *G. lucidum* on some liver enzymes in rats

The effect of sub acute administration of crude methanol extract of wild *G. lucidum* on some liver enzymes is presented in Table 1. Rats treated with 200 mg/kg of the extract showed significant (P < 0.05) increases in ALP on day 14 and 21 when compared with untreated animals. Rats treated with 400 mg/kg of the extract had significant (P < 0.05) increase of ALP on days 7, 14 and 21 compared to pre-treatment values and non-treated control animals. Rats treated with 800 mg/kg showed significant (P < 0.05) decreases in ALP on day 7, 14 and 21 after treatment when compared to values obtained before treatment in untreated control animals.

Rats treated with the extract at doses of 200 and 400 mg/kg had significant (P < 0.05) decrease in AST levels on days 7 and 21 of treatment compared with values obtained before treatment and that of untreated control animals. However, animals that receive the extract at the dose of 800 mg/kg had significant (P < 0.05) increase in AST levels on days 7, 14 and 21 after treatment when compared with values obtained before treatment and that of untreated control animals.

Rats treated with the extract at dose of 200 mg/kg had significant (P < 0.05) decrease in the in the levels of ALT on days 7, 14 and 21 after treatment when compare with pre-treatment and values and that of untreated control animals. Also rats treated with the extract at the dose of
400 mg/kg had significant (P < 0.05) decrease in serum ALT on days 7 and 14 when compared with values obtained from the same animals before treatment and that of untreated control rats.

The mean serum urea concentration showed that treatment with 200 and 800 mg/kg had significant (P < 0.05) increase on days 14 and 21 when compared to pre-treatment value and that obtained from the untreated control animals.

Rats treated with 200 and 400 mg/kg had significantly (P<0.05) increase in levels of serum creatinine on days 14 and 21 compared to pre-treatment value and that from the untreated control animals.

Rats treated with the extract at dose 200 mg/kg had significant (P < 0.05) decrease on day 7 in levels of serum total bilirubin concentration compared to pre-treatment values and that obtained from the untreated control animals. Significant (P < 0.05) increase in serum levels of total bilirubin was observed on day 14 and 21 compared to pre-treatment and values obtained from untreated control animals.

**Effect of sub acute administration of methanol fraction of wild G. lucidum on Liver enzyme**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dose (mg/kg)</th>
<th>Pre – treatment</th>
<th>Treatment (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>14 Mean ± SD</td>
</tr>
<tr>
<td><strong>ALP (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>13.4 ± 3.0 b</td>
<td>13.0 ± 0.1 c</td>
<td>221 ± 2.1 a</td>
</tr>
<tr>
<td>400</td>
<td>68 ± 2.0 a</td>
<td>232 ± 2.1 a</td>
<td>128 ± 3.4 a</td>
</tr>
<tr>
<td>800</td>
<td>34 ± 1.0 a</td>
<td>167 ± 3.0 a</td>
<td>220 ± 0.3 a</td>
</tr>
<tr>
<td>Control</td>
<td>17 ± 0.1</td>
<td>17 ± 0.1</td>
<td>18 ± 6.0</td>
</tr>
<tr>
<td><strong>AST (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>96 ± 3.0 a</td>
<td>96 ± 3.4 a</td>
<td>41 ± 1.1 b</td>
</tr>
<tr>
<td>400</td>
<td>76 ± 2.0 a</td>
<td>89 ± 2.0 a</td>
<td>31 ± 2.1 b</td>
</tr>
<tr>
<td>800</td>
<td>39 ± 0.1 a</td>
<td>67 ± 3.0 a</td>
<td>220 ± 1.3 a</td>
</tr>
<tr>
<td>Control</td>
<td>16 ± 0.3</td>
<td>16.1 ± 0.4</td>
<td>17 ± 0.5</td>
</tr>
<tr>
<td><strong>ALT (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>39 ± 0.1 d</td>
<td>25 ± 0.8 b</td>
<td>25 ± 0.8 b</td>
</tr>
<tr>
<td>400</td>
<td>21 ± 0.3 c</td>
<td>21 ± 2.1 d</td>
<td>17 ± 1.4 b</td>
</tr>
<tr>
<td>800</td>
<td>21 ± 3.4 c</td>
<td>17 ± 3.4 a</td>
<td>21 ± 1.2 d</td>
</tr>
<tr>
<td>Control</td>
<td>32 ± 0.0</td>
<td>34 ± 0.1</td>
<td>35 ± 0.1</td>
</tr>
<tr>
<td><strong>Urea (mmol/L)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>5.4 ± 0.5 d</td>
<td>4.4 ± 0.3 d</td>
<td>6.7 ± 3.1 d</td>
</tr>
</tbody>
</table>

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**DISCUSSION**

The increase in serum alkaline phosphatase (ALP) concentration in this work can be considered as an adaptive response by the rats system to absorbed foreign substance. It may also be due to hepatic damage that could have induced cytochrome P450 induction. This agree with findings of Enullat *et al.* (2010) who reported that increase in serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase can be induced by drugs (which are foreign substances) in rats, dogs and horses. It can also be due to increase in circulating neutrophils as observed by Musakazu *et al.* (2005 and Shamaki *et al.* (2014) demonstrating that increase in levels of serum ALP can be due to neutrophillic leucocytes. (Stewarts, 1974)

In this study, the dose dependent increase in serum aspartate aminotransferase (AST) in rats treated with extract of *G. lucidum* is an indication of liver injury. Serum AST is a non specific liver enzyme (Enullat *et al.*, 2010). Increase in serum concentration of AST has been linked to hepatic pathology (Kashemi-Shirazi *et al.*, 2008), and to other liver related diseases (Enullat *et al.*, 2010). Histopathological findings in this study showed nuclear and cytoplasmic necrotic changes in the liver of rats treated with the extract at high doses and this can probably explain the observed increase in serum ALT. Serum AST which are similarly
reported to be tissue non specific and its activity is found in almost all cells, including red blood cells, but muscle and liver are considered to be organs with high concentration of ALT and AST (Holy, 2003), although the enzyme has a short half life (Perry, 2003), its concentration still remains high even after cessation of treatment. Significant (P < 0.05) increase in ALT concentration has been associated with hepatocellular injury (Enullat et al., 2010) which was observed in this study. This increase was observed to be delayed. Perry, (2003) reported increase in serum ALT with sub lethal hepatocellular injury or necrosis. The increase was delayed to third week of treatment. This agrees with findings of Boll et al (1998) and Roberts et al (1995) who reported delayed increase in serum ALT activity in rats. At lower doses, there was observed decreases in levels of serum AST agreeing with findings of Wasser and Weis 91997) of hepatoprotective effect of G. lucidum extract.

In this study, the serum urea concentration increased significant (P < 0.05) during the second week in animals treated with 800 mg/kg compared to serum levels obtained before treatment and in untreated control animals. This increase is dose dependent. This could be due to diminished glomerular filtration and dehydration (Christopher, 2003).

Similarly, the serum creatinine concentration were observed to significantly (P < 0.05) increase at day 21 of treatment in animals treated with 200, 400 mg/kg of the extracts compared to creatinine levels obtained before treatment and that of untreated control animals. This increase corresponds to increase in levels of urea, which indicates kidney damage. It can also be due to conditioning of the experimental rats and possibly increase in muscular activity. The increase of serum creatinine is associated with renal disease (Christopher, 2003), and may contribute the findings in this study as degenerative (cloudy swelling) and cytoplasmic (necrosis) changes were both observed in histological study. This possibly is due to increase in levels of serum creatinine as observed in this study.

Bilirubin is formed from breakdown products of haemoglobin and to some extent, non haem pophyrins that are mostly produced in mononuclear phagocytes (Perry, 2003). The observed increase in serum bilirubin concentrations, in all the treatment groups during treatment, may be due to the non haem pophyrins no changes were reported in in the levels of haemoglobin concentrations in another study (Shamaki et al., 2014) This increase corresponds to increases in albumin levels in the serum that are reported to be usually bound covalently and irreversibly to serum albumin as biliproteins. Increase in serum bilirubin can also be due to decrease uptake in cholestatic and hepatocellular disease such as congestion, necrosis and
inflammation as histological findings in this study showed this condition in the liver and degenerative changes and necrosis in the kidney of treated rats.

**Recommendation**

High concentration of *G. lucidum* should be avoided especially over a long period of time due to observations from the present study.

**ACKNOWLEDGEMENT**

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