Toxicity studies and body weights changes in Wistar rats following oral administration of methanol extract from indigenous ganoderma sp. in Nigeria

Abstract
The acute and sub acute toxicity studies of methanol extract of Ganoderma lucidum was evaluated in Wistar rats using standard methods. Various doses (200, 400 800 and 3200mg/kg) of the extract were orally administered to Wistar rats over a period of 24 hours in acute toxicity studies, while selected doses of 200, 400 and 800 mg/kg of the extract were orally administered daily, for 21 days to rats according to body weights. Results of findings in both studies showed no apparent clinical sign of toxicity, and no gross lesion was observed in the excised organs (lung, liver, kidney, spleen and heart) of the experimental rats. The experimental rats responded well through increases in body weights throughout the study period. These findings showed safety of the methanol extract of the wild Ganoderma spp. in wistar rats, implying a wide margin of safety following consumption for a long period of time, especially at doses of 200 and 400mg/kg, the rats were equally observed to reverse changes in their physiological values following a withdrawal period of two weeks. This implied the effective biotransformation and elimination of this natural product within a short time period.

Keywords: toxicity, dose, methanol extract, ganoderma lucidum, wistar rats

Introduction
Traditional medicine is in recent times gaining acceptance in the healthcare delivery systems of many countries, with the World Health Organization reporting that over 80% of the world’s population still relies on herbal medicine as their primary source of healthcare, and with millions of Africans of all ages relying on herbal medicines for primary healthcare.1 Which underscores the importance of herbal medicine in healthcare delivery system. Despite these, little effort is being made to apply traditional medicine into Veterinary practice2 beyond its use by herdsmen. Although, traditional medicine can be a source of medication to widen the therapeutic arsenal against range of diseases,3,4 it is important to know that plants produce diverse array of chemical substances that may form the basis for their use in traditional medicine to treat variety of disease conditions, and can therefore, be an enormous potential source of raw material for new chemotherapeutic agents.3 However, these chemicals produced by plants can have other unwanted pharmacological effects on some tissues, haematological parameters and other indices of production. Among a range of plants that are currently being investigated for their medicinal properties is the mushroom, Ganoderma lucidum. The evaluation of toxic activities of this mushroom is important in order to consider safe preparations that can enable the definition of the intrinsic toxicity and the effects the acute and sub acute dose of the mushroom has on its consumers.6 Although, extracts of this mushroom is reported to possess a number of pharmacologically active phytochemicals and elements7 with evidence of a lot of medicinal properties,8,9,10 it was also reported to have unwanted toxicological effects on internal organs such as liver, kidneys, lungs, spleen and the heart.11 Preparations of this mushroom are used as tea or components of immune boosters (immunoboost8) and as other antioxidant supplement. This study is therefore important, to evaluate the acute and sub acute toxic effects of extracts of this mushroom.

Materials and methods
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 in a clean 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 4ºC until required.

Extraction of the wild G. lucidum
The dried powder of Ganoderma was weighed using a metler balance (Toledo-PB 153, Switzerland), and 1.5g 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 24h, using electric evaporator.

Laboratory animals
A total of one hundred and thirty (130) albino rats, three weeks old, of different sexes weighing between 100 and 140 g were used...
for the toxicity studies. The rats were kept in plastic cages with open top, sealed with wire gauze in the Veterinary Physiology laboratory of the Department of Veterinary Physiology, Pharmacology and Biochemistry. Animals were acclimatized to laboratory conditions for 2 weeks before commencement of the experiment. The rats were fed with commercially formulated pelleted broilers feed-Grower mash produced by Vital feeds Company Nigeria Ltd, Bukuru, Jos, Plateau State Nigeria. Clean drinking water was provided ad libitum.

**Determination of body and organ weight changes in rats**

Changes in the body weights of the experimental rats were monitored on weekly basis by weighing each rat daily using a compact scale weighing balance (FEJ-3000B, 3000g capacity, China). Similarly, tissue samples from the lungs, liver, heart, kidney and spleen were collected from sacrificed rats daily, and were weighed using electronic weighing balance (SFE 300, Citizens scale, Adams equipment company limited, China) to determine the organ-body weight relationship.

**Toxicity studies**

**Acute toxicity studies (LD50)**

Thirty rats of both sexes, weighing 112g-140g were used in this study. These rats were divided at random into six groups (A, B, C, D and E and F) of 5 rats each. The rats were allowed to acclimatize for two weeks in clean cages under laboratory conditions. They were fed with commercially formulated pelleted feed (Vital feeds Plc, Bukuru, Jos, Plateau State Nigeria) and clean water, ad libitum. Rats in group A, B, C, D and E were treated with crude methanol extract of *G. lucidum* at 200, 400, 800, 1,600 and 3200mg/kg body weights respectively. Rats in group F served as untreated control and were given distilled water only. All treatments were given orally. The rats were monitored for clinical signs, behavioural changes and death over a period of 24 hours. The LD50 was determined using the Arithmetical methods of Kerber as modified by Aliu and Nwude.

**Table 1 Acute toxicity studies of methanol extract of wild *G. lucidum* in rats (n=5)**

| Group | Dose (mg/kg) | Average body weight (g) | Volume of extract administered (ml) | Mortality | Survivability |
|-------|--------------|-------------------------|------------------------------------|-----------|--------------|
| A     | 200          | 138.7±1.1               | 0.14                               | 0         | 5            |
| B     | 400          | 139.7±2.1               | 0.5                                | 0         | 5            |
| C     | 800          | 113.6±2.4               | 0.4                                | 0         | 5            |
| D     | 1600         | 129.2±1.5               | 1                                  | 0         | 5            |
| E     | 3200         | 138±3.1                 | 1.2                                | 0         | 5            |
| F     | Control      | 132.4±0.8               | 1.2                                | 0         | 5            |

LD50≥3200mg/kg

**Sub acute toxicity studies**

One hundred albino rats of weights between 112g to 140g, and aged four weeks of both sexes were randomly allotted into four groups (A, B, C, D) with twenty five rats in each group. Methanol extract of the wild *G. lucidum* was administered orally at varying doses of 200, 400 and 800mg/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 for five weeks (day 7th, 14th, 21st, 28th and 35th). Five rats from each group were sacrificed every week by severing the jugular vein. Thereafter, 1.5ml of blood collected into bijour bottles from each rat. Collected blood from each rat was divided into two equal parts. One part was used to analyse for haematological parameters, while the other part was allowed to clot and the serum analysed for biochemical parameters.

**Results**

In acute toxicity studies, oral administration of varying doses (200, 400, 600, 1,600 and 3200mg/kg) of methanol fraction of the wild *Ganoderma lucidum* extract in Wistar rats caused itching around the mouth area which caused rats to scratch their mouths with their fore limbs. The observed effect increased with increase in the dose of the extract. The signs were noticed 20 minutes after the administration of the extract. These were observed especially in the rats that were treated with 1600 and 3200mg/kg. These signs were mild in rats given lower doses of 200 and 800mg/kg. There was no mortality recorded in all the treated groups, and all the treated experimental rats survived. (Table 1) in sub acute studies, Wistar rats treated with the extract at doses of 200, 400 and 800mg/kg, did not show any apparent sign of toxicity as that observed in the acute toxicity studies. However the experimental rats in all the groups were observed to feed and drink water ad libitum normally, especially with higher doses of 1600 and 3200mg/kg respectively. These signs (increase in feeding a drinking) were observed between 7-21 days (weeks of treatment) which declined during the withdrawal period at 28-35 days (two weeks withdrawal period) (Table 2).

**Table 2 Effect of sub acute administration of methanol extract of wild *G. lucidum* on body weight in rats**

| Dose of extract (mg/kg) | Weekly body weight changes (g) (Mean±SD) Day |
|-------------------------|---------------------------------------------|
| 200                     | 129.2±9.5 128.6±9.0c 128.5±9.5c 138.7±1.1a 134.0±16.1a 164.3±19.6a |
| 400                     | 103.4±14.9a 105.8±15.2c 109.3±15.1b 119.8±15.6 135.1±11.9 145.2±12.1a |
| 800                     | 119.8±10.1b 120.8±11.8c 126.0±11.9a 139.5±10.7a 138.1±17.0b 148.5±17.1a |
| Control (distilled water) (ml/kg) | 125.0±20.9 123.8±20.5 127.1±20.3 133.5±25.7 146.7±7.8 149.9±20.7 |

*: Cessation of treatment.

Mean with different superscript letters differ significantly (P<0.05).

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Body weight changes

Rats treated with the extract had significant (p<0.05) increase in body weight after 21 days of treatment (Table 2). There were no significant changes in weights of treated rats 2 weeks after the cessation of treatment. The body weights of the rats before treatment were 129±9.5, 103.4±14.9, 119.8±10.1 and 125.0±20.9 for groups A, B, C and D respectively. By 21 days of treatment, the body weights of rats were 138.7±1.1, 119.8±15.6, 139.5±10.7 and 133.5±7.5 in groups A, B, C and D respectively. The pattern of weight gain was not interfered with during the withdrawal of the extract.

Statistical analysis

Data obtained from sub-acute studies was analysed by one way analysis of variance (ANOVA) using Graphpad InStat10 statistical software package to analyse the degree of variance among groups, and “p” values less than, or equal to 0.05 (Turkeys test) was considered significant.

Discussion

The increasing demand for the inclusion of plants extracts and other herbal preparations in both human and animal health programmes necessitate the toxic actions of the plant extract to assess their inherent toxicity levels and the effect of acute overdose. This is to safeguard the safety limits of their use in both animal and man. Acute toxicity studies of the methanol extract of G. lucidum showed no mortality in experimental Wistar rats at high dose of 3600mg/kg when administered orally. Thus, suggesting that the mushroom has low acute toxicity and has wide margin of safety. This finding agree with that of Clark and Clark20 that said substances whose LD50 in rats fall below 50-100mg/kg should be regarded as very toxic, and those with LD50 above 500mg/kg but below 1000mg/kg are classified as being moderately toxic, while substances whose LD50 in rats are above 1000mg/kg are considered safe or of low toxicity. This indicated that the methanol fraction of G. lucidum extracts can be administered orally in rats with some degree of safety. Reports by Dennis21 suggested that absorption of substances orally administered is interfered with in the gastrointestinal tracts, therefore, limiting the absorption rates, hence absence of gross toxicity signs observed. However, Oluba et al.22 Reported non toxic effect of the intra peritoneal (i.p) administration of aqueous extract of G. lucidum in rats, thus, indicating both routes produce no toxic effect of extract of this mushroom in rats. In this study, there was no apparent clinical signs of toxicity observed in both acute and sub acute studies when the extract was orally administered, however, it was observed that feed and water consumption increase within the period of study, thus causing an increase in body weights of experimental rats. This may be due to increase in metabolism and anti oxidant effect of the extract as reported by Oluba et al.22-24 while other reports indicate decrease in weight.25

The significant (P<0.005) increase in body weights of rats observed in both the extracts treated rats and the control groups suggest that the extract has no effects on feed conversion rates in the experimental rats. However, this may be influenced by the presence of steroids and saponins26 and with the reports that saponins can be converted to aglycon sapogenin (a steroid or triterpene)27 which may probably acts centrally and have stimulatory effects on feeding centers in the brain of experimental rats. Body weight changes can occur through alteration in growth especially when they contain agents that modify secretion of growth hormone or somatostatin, or through alteration of hormonal status eg, agents that modify secretion of sex steroids and therefore alter maturational pattern or through changes in neurotransmitters that affect food consumption, such as agents that affect central seratoninergic or dopaminergic systems, reduced palatability of diets containing the experimental compound or through non specific systemic toxicity.27 Hence the increase in feed and water intake observed in the rats, and subsequently, increase in body weights in the extracts treated group compared to control and pre-treatment. Weight gain was not reversed following withdrawal period of two weeks, thus, implying effective feed conversion in the experimental rats. Although there was no apparent gross lesion observed in both studies, the methanol extract was reported to cause histopathological changes in most of the studied organs reported by Oluba et al.15,22 and Sharmaki et al.15 This implies that prolonged administration cum consumption of extract of Ganoderma lucidum at higher doses (>400mg/kg) may show no apparent gross lesion, but can cause damages to some internal organs.

Conclusion/recommendation

It was therefore concluded that methanol extract of G. lucidum did not produce any gross lesion in rats and can be considered safe when administered orally, and recommended that such should be exploited in further research for medicinal purpose. Furthermore, feeding and drinking habits of rats under G. lucidum extract treatment should be carried out to evaluate its veracity and equally presence or otherwise, of possible anti-nutritive elements in the mushroom extracts.

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Conflict of interest

The author declares no conflict of interest.

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