Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Nutritional and Antinutritional Composition Analyses and Toxicity Assessment of *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. in Mice

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ARTICLE DETAILS

Article history:
Received on 01 September 2014
Modified on 22 September 2014
Accepted on 27 September 2014

Keywords:
Proximate composition,
Anti-nutritive factors,
Medicinal mushroom,
Ganoderma lucidum,
Toxicity

ABSTRACT

The nutritional composition and anti-nutritive contents of wild *Ganoderma lucidum* fruit bodies were determined and its aqueous extract assessed for subchronic oral toxicity in mice. The matured fruit body contained per 100 g total carbohydrate (42.10 g), crude fibre (32.10 g), moisture (10.80 g), crude protein (7.18 g), ash (6.02 g) and crude fat (3.50 g). The antinutritive factors content per 100 g were cyanide (0.005 mg), oxalate (0.36 mg) and phytate (0.018). Crude aqueous extract of *Ganoderma lucidum* at 100, 250 and 500 mg/kg body weight for 21 days did not significantly affect the relative liver, kidney and spleen weights compared with control but relative heart weights were significantly higher compared with control in mice administered 100 and 500 mg/kg extract. Hematological indices, serum markers of liver and kidney status and serum lipid profile showed no statistically significant differences in the extract treated mice compared with control. It was therefore concluded that fruit body of *G. lucidum* contained good nutritional profile and its consumption both for nutritional and pharmaceutical purposes in the short-term could be said to be relatively safe.

INTRODUCTION

Ganoderma lucidum (Fr.) Karst (Polyporaceae) is widely known in China as 'the mushroom of immortality'. Among cultivated mushrooms, *Ganoderma* is unique in being consummed not only for its nutritional potential but also for its pharmaceutical value. The fruit bodies are used for the treatment of neurasthenia, deficiency fatigue, insomnia, bronchial cough in elderly people and carcinoma.[1] In China and Japan, *Ganoderma lucidum* are cultivated and utilized as useful source of feed supplement and as medicine for promoting health and immune functions in humans and for prevention and treatment of certain diseases.[2,3]

In Africa, Tanzanian is the only country reported to be actively exploring *Ganoderma* species of mushroom.[4]

In Nigeria, qualitative and quantitative analysis of wild *Ganoderma* species harvested from Vom by Ogbe *et al*.[5] have revealed the high nutritional potential of this medicinal mushroom. However, in a report by Mckenna *et al*.[6], qualitative and quantitative differences have been shown to exist in the chemical composition of *Ganoderma* products due to factors such as strain, origin, extraction process, and cultivation conditions. Thus, the need to investigate the chemical composition of *Ganoderma lucidum* obtained in different regions within the same country or continent was warranted. Moreso, little information is available on toxicity of local *Ganoderma lucidum*. Attempt has been made in the study to analyse fruit bodies of *Ganoderma lucidum* harvested from a rain forest in Ifon (Ondo State) in southwestern Nigeria for nutritional and anti-nutritional composition and to evaluate its crude aqueous extract for toxicity in mice.

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MATERIALS AND METHODS

Fungal Material

Fruit bodies of *G. lucidum* were obtained locally from an open forest in Ifon (Ose Local Government, Ondo State, Nigeria). The mushroom material was identified by a taxonomist in the Department of Plant Science, University of Benin, Benin City, Nigeria; where voucher samples were kept for reference.

Animals and Diet

Five weeks old healthy male Swiss albino mice (*Mus musculus*) between 15 and 22.5 g purchased from the animal laboratory of University of Ibadan, Ibadan was used for the study. The animals were housed in stainless steel cages with raised wire floor in a room with 12 h light/dark cycle and 50-60% relative humidity at a temperature of about 30^0C. The animals were fed standard rat chow (Premier Feeds Mills Co. Ltd., Ibadan). The animals had free access to food and water and were treated according to the International guidelines for the care and use of laboratory animals.[7] They were kept under the laboratory conditions for two weeks before the start of the experiments.

Preparation of Fungal Extracts

Fruit bodies of *G. lucidum* were sorted, washed to remove debris and dust particles and then dried under shade for two weeks. The dried materials were cut into pieces and grinded into powder using mechanical grinder. The powdered sample was kept in airtight container and stored at 4^0C until required for further analysis.

Extraction Procedures

A known quantity (50 g) of the powdered extract was macerated in distilled water and extracted twice, on each occasion with 500 ml distilled water at room temperature for 48 hr with constant stirring. On each occasion the mixture was filtered using Whatman (number 1) filter paper. The combined aqueous extract soluble (dark brown filtrate) was evaporated at 45^0C on water bath to dryness giving 2.25 g yield. The residue obtained was kept in dry sterilized air-tight container at 4^0C.

Proximate Composition Analysis

The proximate composition analysis of the fruit bodies of *Ganoderma lucidum* was carried out using the methods of the A.O.A.C.[8]

Antinutrient Analysis

Determination of Hydrogen Cyanide (HCN)

The procedure described by AOAC [9] was followed to determine the hydrogen cyanide content of the sample. A known quantity (10.0 g) of the powdered mushroom sample was soaked in a mixture containing distilled water (200.0 cm^3) and orthophosphoric acid (10 cm^3). The mixture was left for 12 h to release all bound hydrocyanic acid. A drop each of antifoaming (paraffin) and antibumping agents were added and the solution distilled until 50.0 cm^3 of the distillate was collected. About 20 cm^3 of the distillate was put in conical flask and 40.0 cm^3 potassium iodide (5% ($^w/_v$) solution was added. The resulting mixture was titrated with 0.02 $moldm^{-3}$ silver nitrate ($AgNO_3$) using microburette until a faint but permanent turbidity was obtained (1 cm^3 0.02 $moldm^{-3}$, $AgNO_3$ = 1.08 HCN).

Determination of Phytate

Phytate content was estimated using the method described by Reddy *et al*.[10] A known quantity (4.0 g) of the powdered mushroom sample was soaked in 100 cm^3 hydrochloric acid (2%) for 5 h and then filtered. About 25.0 cm^3 of the filtrate was taken into a conical flask and 5.0 cm^3 of 0.3% ammonium thiocyanate solution was added. The mixture was then titrated with a standard solution of iron (III) chloride until a brownish-yellow colour was formed (persisting for 5 min).

Determination of Oxalate Content

Oxalate content of the sample was determined following the procedure described by Anhwange.[11] A known quantity (1.0 g) of the powdered sample was placed in a 250.0 cm^3 volumetric flask. To this 190.0 cm^3 of distilled water and 10.0 cm^3 of 6 $moldm^{-3}$ HCl were added. The mixture was warmed on a water bath at 90^0C for 4 h; and the resulting digested sample was centrifuged at 2000 g for 5 min and filtered. The fitrate was diluted to 250.0 cm^3 with distilled water. Two hundred aliquot of the diluted filtrate was evaporated to 25.0 cm^3 and the resulting brown precipitate was filtered off. The filtrate was then titrated with concentrated ammonia solution until a faint yellow colouration was obtained using methyl orange as indicator. The solution was then heated to 90 ^{0}C and the oxalate was precipitated with 10.0 cm^3 of 5% calcium chloride solution. The solution was left overnight and then diluted to 125.0 cm^3 with distilled water after warming to 90^0C and titrated against 0.05$moldm^{-3}$ $KMnO_4$.

Subchronic Oral Toxicity Test

Thirty-two (32) male Swiss albino mice (15 – 22.5 g) were divided into two groups (n=8)

designated: Control and experimentals. The experimental group consisted of three sub-groups designated: AQ_{100}, AQ_{250} and AQ_{500}. Animals in control group were administered 1.2 ml distilled water while those in experimental groups, AQ_{100}, AQ_{250} and AQ_{500} were administered 100, 250, and 500 mg/kg of the aqueous extract, respectively. Extract was administered once daily for 21 days using intragastric tube. Mice in each group were observed daily for signs of poisoning. Food and water intake were recorded weekly for each group. All experimental protocols complied with NIH guidelines for care and use of laboratory animals [7].

Sample Collections and Processing of Samples

At the end of the treatment period, mice in each group were fasted overnight, weighed and then sacrificed by cervical dislocation. Blood was collected through their jugular vein into plain, sterile bottles. The blood samples were allowed to clot and then centrifuged at 3,000x g for ten minutes at room temperature to obtain the sera. The sera samples were collected by aspiration using a Pasture pipette into sterile bijou bottles and frozen until required for analysis, which was done within 72 h.

Organ Body Weight Ratio

Immediately after sacrifice vital organs such as liver, kidneys, heart and spleen were quickly excised, freed of fat, blotted with clean laboratory tissue paper and then weighed. Organ to body weight ratio was determined by comparing the weight of each organ with the final body weight of each mouse.

Determination of Biochemical Parameters

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltranspeptidase (γ-GT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activities and serum albumin, total protein, urea nitrogen, creatinine, as well as triacylglycerol (TAG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) were assayed using an auto-analyzer (Hitachi 7060, Hitachi, Tokyo, Japan).

Statistical Analysis

Data are represented as means ± SEM of eight independent determinations. The mean values of control and test groups were compared using one way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) performed using SPSS 17 software. $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Proximate composition analysis of *Ganoderma lucidum* as obtained from this study showed that its fruit body is richest in total carbohydrate (42.10%), followed by crude fibre (32.10%), moisture (10.80%), crude protein (7.18%), ash (6.02%) and lastly crude fat (3.50%) (Table 1). These values were similar to those obtained by Ogbe *et al*. [5] for analysis of wild *Ganoderma lucidum* obtained from one the north-central states in Nigeria (Nasarawa State). In addition, our results are in consonance with data obtained by authors from other regions.[12-14] However, the crude protein and total carbohydrate contents of *G. lucidum* fruit body as revealed in this study is low compared with that reported by Wasser.[15] The contradition may be attributed to factors such as origin, strain, extraction process and cultivation conditions.[13,14] The rich nutritional profile of the fruit body of *G. lucidum* as revealed in this study shows the potential of the mushroom as possible source of good nutrition especially in cardiovascular health due to its low fat content.

Table 1: Proximate composition analysis and antinutritional composition of dried fruit body of *Ganoderma lucidum*

	Constituents	Quantity
Proximate composition	Moisture (%)	10.80
	Ash (%)	6.02
	Crude protein (%)	7.18
	Crude fat (%)	3.50
	Crude fibre (%)	32.10
	Total carbohydrate %)	40.40
Antinutritive factors	Cyanide (mg/100 g)	0.005
	Oxalate (mg/100 g)	0.36
	Phytate (mg/100 g)	0.018

The levels of the anti-nutritive factors detected in fruit body of *Ganoderma lucidum* as presented in Table 1 revealed low levels of cyanide (0.005 mg/100 g), oxalate (0.36 mg/100 g) and phytate (0.018 g/100 g). From these values it is evident that though *Ganoderma lucidum* contained the three anti-nutritive factors evaluated in this study but at a considerably low level hence utilization of wild *Ganoderma lucidum* may not lead to health problems in rodents and other mammals. Phytate in food binds essential minerals in the digestive tract, resulting in

mineral deficiencies.[16] Moreso, some of these anti-nutrients (phytate, oxalate and tannins) can be reduced by proper food processing techniques.[17] Soaking the mushroom in boiled water during aqueous extraction would further reduce the levels of these anti-nutritive factors.

No noteworthy changes in general condition were noted in any group during the treatment period. None of the animals died during any part of the study. There were no differences in body weight gain and food consumption in experimental groups compared with the control. Crude aqueous extract of *Ganoderma lucidum* at 100, 250 and 500 mg/kg body weight administered to mice for 21 days did not significantly affect the liver, kidneys and spleen weights relative to body weight when compared with untreated control (Table 2). The heart/body weight ratios were significantly higher ($p < 0.05$) in treated mice compared with control except for mice administered 250 mg/kg where the relative heart weights were not statistically different ($p > 0.05$) from control. This observation is consistent with the report of Sasidharan *et al*. [18] who had earlier observed a non significant change in biochemical or behavioural abnormality following exposure of mice to a single dose of 2000 mg/kg methanolic extract of *Ganoderma boninense*.

Table 2: Organ body weight ratio of mice administered *Ganoderma lucidum* extracts and chloroquine for 21 days

Treatment groups	Liver	Kidney	Heart	Spleen
Control	0.091±0.001[a]	0.016±0.001[a]	0.006±0.000[a]	0.007±0.001[a]
AQ_{100}	0.084±0.002[a]	0.016±0.001[a]	0.008±0.001[b]	0.008±0.000[a]
AQ_{250}	0.083±0.003[a]	0.016±0.000[a]	0.006±0.000[a]	0.008±0.001[a]
AQ_{500}	0.087±0.002[a]	0.017±0.000[a]	0.008±0.000[b]	0.009±0.000[a]

Results are represented as Means SEM of eight determinations. Values in the same row carring different superscripts are significant ($p<0.05$).
AQ_{100}, Mice administered 100 mg/kg extract; AQ_{250}, Mice administered 250 mg/kg extract; AQ_{500}, Mice administered 500 mg/kg extract

Hematological indices in *G. lucidum* treated mice and control mice were not significantly different (Table 3). Hemoglobin (Hb), packed cell volume (PCV), platelets (plt) and white blood cell (WBC) levels show similar pattern in both experimental and control mice. The hematopoietic system has been proven to be very sensitive to toxic compounds and thus provides a good index assessing physiological and pathological status of both animals and humans.[19] The present study indicated that a 21-day administration of aqueous extract of *G. lucidum* to mice produced no treatment-related changes in hematological parameters in mice. It is thus infered that aqueous extract of *G. lucidum* did not affect hematopoiesis and leucopoiesis in mice. The orally administered doses of the extract was non-toxic and thus did not interfere with the production of circulating red blood cells, white blood cells and platelets.

Table 3: Hematological profile of mice administered *Ganoderma lucidum* extracts and chloroquine for 21 days

Treatment groups	HB (g/dl)	PCV (%)	PLT (x 100/cmm)	WBC (x 100/cmm)
Control	149.95±17.1[a]	40.1±2.3[a]	13.3±1.3[a]	120.1±12.6[a]
AQ_{100}	138.2±8.0[a]	38.6±4.1[a]	15.1±3.3[a]	124.0±2.5[a]
AQ_{250}	141.7±10.2[a]	38.5±2.2[a]	16.5±1.3[a]	128.5±10.1[a]
AQ_{500}	140.3±11.7[a]	37.3±2.0[a]	14.9±1.6[a]	127.9±7.2[a]

Results are represented as Means SEM of eight determinations. Values in the same row carring different superscripts are significant ($p<0.05$).
AQ_{100}, Mice administered 100 mg/kg extract; AQ_{250}, Mice administered 250 mg/kg extract; AQ_{500}, Mice administered 500 mg/kg extract

Serum activities of ALT, AST, ALP and LDH showed no statistically significant difference in the extract treated mice compared with control. However, serum γ-GT levels showed slight significant decreases in the extract treated mice compared to control (Table 4). Measurement of serum enzyme activity provides a vital tool in clinical diagnosis because it gives information on the effect and nature of pathological damages to tissues. Increased serum levels of enzymes such as ALT, AST, ALP and γ-GT could be indicative of damage to plasma membrane which may lead to a compromise on membranal integrity.[20] Such alterations may also lead to leakage from hepatocytes and possible damage which might have resulted from change in membrane permeability.[21] ALP and γ-GT are membranous enzymes and are thus employed as marker to determine membrane integrity.

Table 4: Serum activities of some tissue specific enzymes in mice administered *Ganoderma lucidum* extracts and chloroquine for 21 days

Treatment groups	ALT (U/mg protein)	AST (U/mg protein)	γ-GT (U/mg protein)	ALP (U/mg protein)	LDH (U/mg protein)
Control	0.32±0.007[a]	0.43±0.009[a]	0.21±0.008[a]	0.58±0.01[a]	1.13±0.10[a]
AQ_{100}	0.33±0.017[a]	0.42±0.008[a]	0.19±0.006[b]	0.60±0.01[a]	1.18±0.05[a]
AQ_{250}	0.32±0.011[a]	0.42±0.010[a]	0.19±0.004[b]	0.59±0.01[a]	1.21±0.10[a]
AQ_{500}	0.33±0.008[a]	0.43±0.005[a]	0.19±0.008[b]	0.62±0.02[a]	1.25±0.10[a]

Results are represented as Means SEM of eight determinations. Values in the same row carring different superscripts are significant ($p<0.05$).
AQ_{100}, Mice administered 100 mg/kg extract; AQ_{250}, Mice administered 250 mg/kg extract; AQ_{500}, Mice administered 500 mg/kg extract

Table 5: Serum albumin, total protein, urea and creatinine concentrations and A/G ratio in mice administered *Ganoderma lucidum* extracts and chloroquine for 21 days

Treatment groups	Albumin (mg/dl)	Albumin/globin	Total protein (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	15.2±2.0[a]	1.18±0.01[a]	48.3±5.2[a]	0.30±0.008[a]	12.3±1.5[a]
AQ_{100}	16.33±1.33[a]	1.13±0.01[a]	45.51±3.3[a]	0.27±0.009[b]	14.1±1.3[a]
AQ_{250}	16.5±1.5[a]	1.15±0.01[a]	47.2±1.5[a]	0.28±0.009[b]	13.8±1.8[a]
AQ_{500}	18.15±1.31[a]	1.18±0.01[a]	47.5±2.1[a]	0.27±0.008[b]	12.5±2.5[a]

Results are represented as Means SEM of eight determinations. Values in the same row carring different superscripts are significant ($p<0.05$).
AQ_{100}, Mice administered 100 mg/kg extract; AQ_{250}, Mice administered 250 mg/kg extract; AQ_{500}, Mice administered 500 mg/kg extract

Table 6: Serum lipid profile in mice administered *Ganoderma lucidum* extracts and chloroquine for 21 days

Treatment groups	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)
Control	12.35±1.33[a]	10.2±2.2[a]	6.62±1.11[a]	1.22±0.01[a]
AQ_{100}	12.75±1.0[a]	12.5±1.5[a]	7.87±1.25[a]	1.5±0.01[b]
AQ_{250}	14.33±1.7[a]	11.8±1.3[a]	7.43±1.15[a]	1.53±0.02[b]
AQ_{500}	15.12±1.3[a]	12.5±1.3[a]	7.88±1.12[a]	1.55±0.01[b]

Results are represented as Means SEM of eight determinations. Values in the same row carring different superscripts are significant ($p<0.05$).
AQ_{100}, Mice administered 100 mg/kg extract; AQ_{250}, Mice administered 250 mg/kg extract; AQ_{500}, Mice administered 500 mg/kg extract

Distruption in the activity of these enzymes confirms compromised membranal integrity. Results from the present study show no disparity in serum ALP and γ-GT acivities between control and treated mice, this could suggest that the extract at doses examined did not cause any perturbation in normal membrane integrity and function. ALT, AST and LDH on the other hand are cytosolic enzymes and are normally localized within the cells of the liver, heart, kidney muscles and other organs.[22] These enzymes can be used to assess damage to the liver and heart (cytolysis). The non-significant change in ALT, AST and LDH activities between treated and untreated mice in this study further reinforce the fact that administration of aqueous extract of *G. lucidum* (100, 250 and 5000 mg/kg bw) produced no distruption in membrane integrity. *G. lucidum* extracts have been reported to show a very low toxicity in animal experiments by several authors.[13,14] In a study by Chiu *et al*.[23] to ascertain the toxicity of wild *G lucidum* fruit bodies harvested in a rural area of Hong Kong in mice, no evidence of acute toxicity nor was serum content of urea, ALT and AST significantly different compared to controls.

No observable significant alteration ($p>0.05$) was noticed in serum albumin, total protein and album/globin ratio between treated and untreated mice (Table 5). Serum concentrations of total protein, albumin and globin may indicate

the state of the liver and the type of damage.[24] The non-significant change in serum albumin, total protein and albumin/globin ratio between treated and untreated mice also add credence to the fact that aqueous extract of *G. lucidum* is likely to be hepatotoxic to mice at doses examined. Serum urea concentration was significantly lower ($p < 0.05$) in the extract treated groups compared to control while serum creatinine concentration shows no significant ($p > 0.05$) between control and experimental groups. This is indicative of the fact that the extract did not interfere with kidney function in mice.

Serum triglycerides, total cholesterol and LDL-cholesterol (LDL) concentrations showed no significant alteration ($p>0.05$) between treated and untreated mice (Table 6). On the other hand serum HDL-cholesterol (HDL) was significantly higher ($p<0.05$) in treated mice groups compared with untreated. Distruption or alteration in body major lipids such as triglycerides, cholesterol and cholesterol lipoproteins (LDL and HDL) can provide useful information on lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated coronary heart diseases. Triglycerides, LDL and HDL are associated with lipolysis, plasma cholesterol transport and atherosclerotic tendency, respectively.[25] The lack of significant alteration in TG, TC and LDL levels between treated and untreated animals in this study indicate that aqeous extract of *G. lucidum* did not affect normal lipid metabolism in mice at all doses investigated. However, the increase in serum HDL in treated animals could be suggestive of the potential of the extract in preventing vital body tissues such as the heart from being overloaded with cholesterol since HDL participate in cholesterol from the tissues back to the liver (reverse cholesterol transport, RCT).

In addition, histological examinations of the liver and kidney, as well as weights of vital organs of the extract treated mice were not significantly altered compared to control (untreated mice group).[23]

CONCLUSION

Data generated from this study revealed that fruit body of *G. lucidum* contained good nutritional profile and its consumption both for nutritional and pharmaceutical purposes in the short-term could be said to be relatively safe.

CONFLICT OF INTEREST

The authors do not have a direct financial relationship with the commercial identity mentioned in this paper.

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