



## RESPONSE OF ORGAN AND BODY WEIGHT IN ALBINO RATS TO ORAL ADMINISTRATION OF METHANOL EXTRACT FROM INDIGENOUS *GANODERMA SP. FROM NIGERIA*

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### ABSTRACT

Organ weight analysis is an important endpoint in toxicological studies for identification of potentially harmful effects of xenobiotics and plant crude extracts. Differences in body weights between groups sometimes may be accompanied by differences in organ weights between these groups. In this study, the relative organ/body weight relationship was examined in 100 three weeks old albino rats of both sexes and weighing 100-140 g following 21 daily oral administration of methanol extract of *Ganoderma spp.* The rats were divided into four groups (A, B, C and D), of 25 rats/group. Doses of 200 mg/kg, 400 mg/kg and 800 mg/kg (constituted from a stock of 2g/100l) of the extract were administered to groups A, B and C respectively within the study period, and rats in group C served as control and were provided water *ad libitum*. Results from the study showed that methanol extract of *G. lucidum* has no effect on relative organ weights of the rats in all the treatment groups compared to control. This suggest that the extract can be tolerated by the body of rats, be fully biotransformed and excreted with relative safety, thereby making the extract of *G. lucidum* a potential source of drug ready for exploitation in the healthcare system.

**KEYWORDS:** *Ganoderma spp.*, methanol extract, relative organ weight, body weight, relationship.

### INTRODUCTION

The mushroom (*Ganoderma lucidum*) was named *Ganoderma* for more than 2,000 years ago by Karsten (1881). It is known in Chinese as “*Lingzhi*”, while it is called “*Reishi*” in Japanese, and “*Hangul*” or “*Yeong-ji*” by the Koreans.<sup>[18]</sup> The English names are known variously as “*glossy Ganoderma*”, “*magic fungus*” and “*marvellous fungus*”.<sup>[11,6]</sup> It is called locally in Nigeria as “*Leman kwado*” in Hausa, “*Koromje pabi*” in Fulfulde, “*Kulongu*” or “*Tumbal kokoye*” in Kanuri, “*Erou*” in Igbo and “*Olu*” or “*Osun*” in Yoruba.

Wild *Ganoderma lucidum* preparations are used in Oriental traditional medicine as antimicrobials. Examples are anti smallpox mushroom, antibiotic mushroom, cytotoxic mushroom and also libido

enhancing mushroom, and recently it was reported to have lowering effect on blood glucose and lipid profiles in Wistar rats.<sup>[14]</sup> Compounds in *G. lucidum* are very complex with 25 amino acids,<sup>[20]</sup> and at the moment, a preparation as medicinal tea that are constantly consumed in some areas of Nigeria. There is growing interest in the use of both edible and medicinal mushroom for food and medicine in Africa and Europe.<sup>[8]</sup>

Report of research findings indicates that the mushroom extract is not showing any gross toxicity<sup>[17]</sup> and that it has some hepatoprotective effects on the liver,<sup>[21,8,10,4]</sup> however, some degree of histopathological toxicity changes were observed in some of these organs.<sup>[16]</sup> However, the ability to assess the effect of xenobiotics

on specific organs is an important requirement in toxicological studies, though faulty conclusions may be reached based on organ weight ratio.<sup>[1]</sup>

Therefore, there is the need to study the effect of methanol extract of this mushroom on body weight-organ ratio using the albino rats as a model, especially when we consider the importance of organ weighing in all specie in multiple doses general toxicology studies that can last between 7 days to 1 year duration.<sup>[12]</sup> Such findings will enable determination of human equivalent dose that may have less toxic effect.

## MATERIALS AND METHODS

### Sample collection

Fresh fruiting part of *Ganoderma spp.* was harvested from Lafia, Nassarawa State in North-central Nigeria during the rainy season (August-September) and it was transported using a clean polythene bag. The mushroom was identified and authenticated at the department of Botany, University of Maiduguri, Nigeria. It was then air-dried in the Laboratory. The dried mushroom was then ground to fine powder using clean pestle and mortar, and this powder was stored in an air tight glass jar at 4°C until required for use.

The air-dried *Ganoderma spp.* powder weighing 1.5 kg was introduced into soxhlet chamber extractor and 7.5 liters of methanol added and extracted at 40°C for 24 hour to obtain methanol solvent extract fraction.

The filtrate was then evaporated within 24 hours using rotator electric evaporator as described by.<sup>[19]</sup> The test, colour and yield of the crude methanol extract were evaluated. This was properly labeled and stored in a glass jar at room temperature until use.

### Preparation of the methanol extract

The prepared dried *Ganoderma spp.* was weighed 1.5 kg, using a metler balance (Toledo-PB 153, Switzerland) and placed in a thimble then into a soxhlet chamber, to this was added 7.5 liters of Methanol and the mixture was steamed at 40°C for 24 hours until the methanol fraction was completely extracted, the filtrate was evaporated within a period of 24 hours, using electric evaporator. A bitter tasting, chocolate colored, sticky extract with a pH of 6.9 and which dissolves in water to give a greenish-yellow colour was realized from the methanol fraction of the mushroom.

### Experimental animals

One hundred (100) three weeks old albino rats of both sexes and weighing between 100-140 grammes were used. The rats were divided into four (4) groups (A, B, C, D,) comprising twenty five rats each, and varying doses of the crude methanol extract of *Ganoderma spp.* was administered orally, to three (3) groups; A, B, C, while group D was administered distilled water for a period of

the experiment. Internal organs such as lungs, liver, kidney, heart and spleen collected by sacrificing five (5) rats/week from each sacrificed rat before treatment and on day 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>th</sup> days respectively and on 28<sup>th</sup> and 35<sup>th</sup> (withdrawal period).

### Relative organ/Body weight determination

Live weights of the experimental albino rats were done using a metler balance prior to oral administration of various doses of the methanol extract of *Ganoderma spp.* and at point of sacrifice (21 days). The internal organs collected on the day of animal sacrifice were cleaned of fats and other mesentery, and absolute weights of these organs were weighed using a metler weighing balance. Relative organ weights in relation to body weights were determined by methods of<sup>[12]</sup> using the formula:

$$\text{Relative Organ Weight (ROW)} = \frac{\text{Absolute organ weight (g)} \times 100}{\text{Body weight at sacrifice (g)}}$$

### Statistical analysis

The data obtained from the study was analyzed by one way analysis of variance (ANOVA) using<sup>[3]</sup> computer software package. "P" values less than or equal to 0.05 was considered significant.

## RESULTS AND DISCUSSION

The results of this findings (Table 1) showed significant ( $p < 0.05$ ) increases in organ's (liver, kidney, spleen, lungs and heart) weights from day 7, and continuously to withdrawal period at 200 mg/kg when these values were compared to day 0. While at 400 mg/kg, the lungs showed significant ( $p < 0.05$ ) increases in weights from day 7 up to withdrawal period, while at 800 mg/kg, the spleen showed significant ( $p < 0.05$ ) increases in weights on days 7 and 14, and the withdrawal periods of day 28 and 35 respectively.

These findings further confirm the hepatoprotective activity of *Ganoderma spp.* extract<sup>[9,20,21]</sup> and in addition, also imply that other internal organs are also protected from effects of oxidants and their function enhanced, especially when consumed at 200 mg/kg.

**Table 1: Effect of prolonged administration of crude extract of *Ganoderma spp.* on relative organ weight (ROW) (g) in Albino rats.**

Grp.	Dose (mg/kg)	Body weights (g)	Organ	ROW (g/wk)					Withdrawal periods	
				0	7	14	21***	28	35	
A	200	138.7 ± 1.1	Liver	1.6±0.1	3.7±0.3*	2.7±0.8*	3.0±0.6*	4.3±0.3*	4.3±0.1*	
			Kidneys	0.2±0.1	0.4±0.2*	0.2±0.6	0.3±0.5	0.4±0.3*	0.4±0.8*	
			Spleen	0.2±0.3	0.4±0.3*	0.4±0.5*	0.4±0.5*	0.5±0.2*	0.5±0.3*	
			Lungs	0.6±0.2	0.8±0.4*	0.8±0.6*	0.7±0.4*	1.2±0.3*	1.2±0.5*	
			Heart	0.2±0.1	0.4±0.4*	0.4±0.3*	0.5±0.4*	0.4±0.5*	0.4±0.4*	
B	400	119.8±15.6	Liver	2.6±0.4	3.5±0.5	2.8±0.4	4.2±0.6	3.5±0.1	3.3±0.3	
			Kidneys	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.6	0.4±0.1	0.3±0.2	
			Spleen	0.3±0.1	0.3±0.3	0.3±0.4	0.3±0.4	0.6±0.5	0.4±0.5	
			Lungs	0.4±0.3	0.8±0.6*	0.9±0.6*	0.7±0.2*	0.8±0.5*	0.7±0.3*	
			Heart	0.3±0.3	0.3±0.4	0.3±0.5	0.4±0.4	0.3±0.6	0.3±0.3	
C	800	139.5±10.7	Liver	2.7±0.2	2.4±0.5	2.7±0.4	2.7±0.4	2.9±0.3	2.7±0.5	
			Kidneys	0.3±0.2	0.3±0.1	0.3±0.5	0.3±0.7	0.4±0.4	0.3±0.6	
			Spleen	0.2±0.4	0.5±0.1*	0.4±0.3*	0.2±0.4	0.5±0.6*	0.5±0.2*	
			Lungs	0.7±0.3	0.7±0.4	0.9±0.9	0.3±0.2	0.9±0.5	0.7±0.3	
			Heart	0.3±0.5	0.3±0.7	0.4±0.8	0.3±0.5	0.4±0.8	0.3±0.7	
D	Control (distil water)	133.5 ± 7.5	Liver	3.4±0.6	2.4±0.4	2.9±0.5	4.4±0.2	3.4±0.3	3.6±0.2	
			Kidneys	0.7±0.3	0.2±0.3	0.3±0.6	0.3±0.3	0.3±0.7	0.3±0.6	
			Spleen	0.3±0.1	0.3±0.4	0.3±0.5	0.5±0.5	0.4±0.4	0.3±0.2	
			Lungs	0.8±0.3	0.6±0.4	0.6±0.4	0.6±0.6	0.7±0.3	0.8±0.1	
			Heart	0.4±0.2	0.2±0.3	0.2±0.5	0.4±0.7	0.4±0.6	0.4±0.3	

\*\*\* = termination of extract administration, ROW=Relative organ weight

\*= significant increases ( $p < 0.05$ ) in organ compared to day 0.

Although there were variations in the doses, body weights between treatment groups and also between controls, there appeared to be difference in the internal organ weights under examination. However, Alteration in body weight changes can occur through alteration in growth hormones that affect food consumption or through non specific systemic toxicity.<sup>[1]</sup> The lungs in this study appeared more affected by the extract especially at doses of 200 mg/kg and 400 mg/kg with increase in weight from day 7 of the experiment through to withdrawal period, however, the increases in organ weights, when compared to day 0 may be attributable to improper administration of the extract in these rats, and may also correspond to increase in body weights of the experimental rats as shown by similar increases in control group, which agrees with findings of<sup>[17]</sup> and that of<sup>[13]</sup> who reported corresponding increase in internal organ weight being proportional to increases in body weights of male rats, and<sup>[22]</sup> who reported that *Ganoderma lucidum*. extract ameliorate nausea, vomiting and food intake dose-dependently in rats. These increases may be due to increase in metabolism and the anti oxidant effects<sup>[9,10,4]</sup> of the mushroom extract hence, increase in feed conversion rates.

The methanol extract of the *Ganoderma spp.* appeared well tolerated by the liver and kidneys in all the treatment groups as observed with no increases in their absolute and relative weights, confirming findings of,<sup>[9]</sup> who reported no significant difference in the absolute

and relative weights of the liver, heart, kidneys and spleen thus implying that the extract is well biotransformed in the liver and effectively excreted through the kidneys from the body of the rats. This may probably explain the non alteration of some enzymes in the liver and kidneys in earlier studies by<sup>[8,15]</sup> However, although there appeared to be no significant change in the relative organ weight,<sup>[23]</sup> reported cytotoxic effect of triterpenoids extract from *Ganoderma linghzi* against cancer cell lines; this may have effect on surrounding tissues. It was similarly reported by<sup>[16]</sup> of histopathological findings on these organs (liver, kidney, spleen, lungs and heart) following 21 days of oral administration of *G. lucidum* methanol extract, thus making consecutive consumption of the mushroom for a long period of time to have unwanted effects, thus disagreeing with findings of<sup>[2]</sup> who reported significant decrease in body weights of female rats after 15 days of administration of hot water extract of *Ganoderma lucidum*.

The spleen and the heart are organs concerned with blood function, while the spleen serve as extra medullary source of blood when there is shortage in circulation, the heart pumps the blood into circulation, the weights of these organs are not affected when compared to control and result from day 0, thus further explaining non change in hematological parameters reported by<sup>[21,14]</sup> impliedly, suggesting that the extract when administered orally in albino rats did not compromise the functions of these

organs. The corresponding increase in organ/body weight relationship may also be due to increase cellular growth in the respective tissues in the absence of traumatic injury, this is because reports by<sup>[7]</sup> suggest that *reishi* can immediately promote cell growth to repair acute injury caused by trauma due to xenobiotics.

### CONCLUSION AND RECOMMENDATION

The study demonstrated that methanol extract of *this wild Ganoderma spp.* has no gross effect on the selected tissues under study and is well tolerated by these organs when administered orally

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