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EFFECT OF PROLONGED ADMINISTRATION OF METHANOLIC EXTRACT OF *GANODERMA LUCIDUM* ON BLOOD GLUCOSE LEVEL IN NORMAL ALBINO RATS

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ABSTRACT

The effect of methanolic extract of *Ganoderma lucidum* on blood glucose level was investigated in normal albino rats by enzymatic oxidation method. Doses of 200 mg/kg, 400 mg/kg and 800 mg/kg were administered to albino rats in groups A, B and C while rats in group D were used as control and administered with water. The extract was orally administered consecutively for 21 days, and two weeks withdrawal period was also monitored. Serum sample was collected from the experimental rats after sacrificing five rats weekly per each group. Pre-treatment, treatment and withdrawal values were analysed and compared to control. Blood glucose level was found to significantly ($P<0.05$) decrease within the treatment period compared to pre-treatment and control suggesting possible hypoglycemic effect of the methanolic extract of wild *Ganoderma lucidum* which may be used in the management of disease such as diabetes mellitus.

Keywords: *Ganoderma lucidum*, Methanol extract, Blood glucose, Albino rats.

INTRODUCTION

Herbs from plant sources were used as mainstream remedy for all ailments, they are rich sources of secondary metabolites which are the potential sources of new drugs [1], and vast majority of our population particularly, those living in the rural areas depend largely on herbal remedies for the treatment of diseases [2].

The world's populations (80 %) were estimated to rely mainly on traditional therapies which involves the use of plant extracts or their active substances [3]. This cause increase awareness to traditional medicine in the healthcare delivery system of human and animal populations in both developing and developed countries. This stimulated efforts in different countries including Nigeria, to conduct researches into traditional medicine and to integrate it with modern orthodox medicine. This will go a long way in reducing dependency of many African countries on imported synthetic drugs [4].

Generally, edible fungus is considered as food substances and little attention is given to their medicinal values. One of such fungi is the wild mushroom (*Ganoderma sp)* which grows at the base of dead woods and logs of deciduous plants. The aim of this study is to investigate the effect of *Ganoderma lucidum* extract on blood glucose level in albino rats.

MATERIAL AND METHOD

Mushroom sample collection and identification

Fresh fruiting part of the wild mushroom (*Ganoderma sp)* was harvested from Lafia, Nassarawa State in North-central Nigeria during the rainy season (August-September, 2011) and it was transported to the laboratory placed in a clean bag. The mushroom was identified and authenticated by a mycologist at the Department of Botany, University of Maiduguri, Nigeria. It was then air-dried in the Laboratory and ground to fine powder using clean mortar and pestle, and this powder was

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stored in an air tight glass jar at room temperature until required for use.

Mushroom extraction

The air-dried *Ganoderma sp.* powder weighing 1.5 kg was introduced into soxhlet chamber extractor and 7.5 Litres of methanol added and extracted at 40^0C for 24 h to obtain methanolic solvent extract fraction.

The filtrate was then evaporated *in vacuo* using rotatory electric evaporator as described by Trease and Evans (1997). The taste, colour and yield of the crude methanol extract were evaluated. The crude extract was properly labeled and stored in a glass jar at room temperature until use.

Preparation of methanolic extract of the wild *Ganoderma lucidum*

The prepared dried *Ganoderma* powder 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 litres of 99 % methanol and the mixture was steamed in water bath at 40^oC for 24 h until the methanol fraction was completely extracted, the filtrate was evaporated within a period of 24 h, using electric evaporator. A bitter tasting, chocolate coloured; sticky extract with a pH of 6.9 was obtained. The crude methanol extract dissolves in water to give a greenish-yellow colour solution of the methanol fraction of the mushroom. This was weighed, and the yield was calculated (79.5 g (5.3 %). By subtracting the final weight of the obtained extract form the initial weight, and multiplying the result by 100.

$$\% \text{ yield} = W_1 - W_2 \times 100$$

Where W_1 initial weight

W_2 =final weight

Doses of 200 mg/kg, 400 mg/kg and 800 mg/kg were prepared from stock preparation of 200 mg/ml and consecutively administered to rats in the treatment groups for a period of 21 days. Two (2) weeks withdrawal period was also monitored, and results of treatment were compared to pre-treatment and control values.

Laboratory Animals

A total of one hundred (100) three weeks old albino rats of different sexes weighing between 100 g-140 g were used for this study. The rats were kept in plastic cages with open top, sealed with wire gauze in the Physiology and Pharmacology laboratory of the Department of Veterinary Physiology, Pharmacology and Biochemistry. They were divided into four (4) groups (A, B, C and D) of 25 rats/group and kept for two weeks to acclimatize before commencement of the experiment. Rats in groups A, B and C were used as treatment group while rats in group D were considered as control. The albino rats were fed with commercially formulated pelletized broilers feed-Grower mash produced by Vital feeds Nigeria Ltd, Bukuru, Jos, Plateau State Nigeria. Clean drinking water was provided *ad libitum.*

Determination of serum glucose

Serum blood glucose in the treated rats was quantitatively determined *in vitro* by enzymatic oxidation method as described by Tietz (1990) [5]. The method is based on the principle that glucose under enzymatic oxidation in the presence of glucose oxidase (GOD) produces gluconic acid and hydrogen peroxide. This hydrogen peroxide reacts with phenol and 4-aminophenazone, catalyzed by peroxidase (POD) to form a red – violet quinonoimine dye as an indicator, and water.

Reagents for the test are constituted by preparing a fresh buffer solution (pH 7.0) by adding 0.1 mmol of phosphate buffer and 11 mmol of phenol into 1 liter of distilled water (R1a), while glucose oxidase-phenol, 4-aminophenazone reagent (GOD-PAP) is prepared by addition of 0.77 mmol of 4-aminophenazone to 1.5 kilo micron of glucose oxidase and 1.5 kilo micron of peroxidase in 1 liter of distilled water. Ready to use glucose is use as standard. These reagents were obtained from RANDOX *CE* Laboratories, Ardmore, United Kingdom, and were prepared and stored at 15^0C- 25^0C 30 minutes before use [6].

The test was performed by mixing 0.01 ml of the serum sample and 1 ml of R1 and incubated for 10 minutes at 37^0C. Using spectrophotometer, the absorbance of the standard ($A_{standard}$) and that of the sample (A_{sample}) was read from a 1 cm cuvette at a wavelength of 500 nm, against the reagent blank within 60 minutes. Glucose concentration was calculated by using the formula:

$$\text{Glucose concentration} = \frac{A_{sample}}{A_{sample}} \times \text{standard conc. (mmol/L or g/dl)}$$

RESULTS

The mean serum glucose concentration (mmol/L) is presented in Table 1. Rats in group A treated with 200 mg/kg of the extract showed an initial increase (0.8 ± 3.4) on day 7 compared to control (0.5 ± 5.0) and a significant ($P<0.05$) decrease on day 14 and 21 (0.4 ± 7.1 and 0.4 ± 2.1) compare to pre-treatment values (0.6 ± 4.3) and controls (1.1 ± 10.2 and 1.6 ± 9.1). Rats in group B showed significant ($P<0.05$) decrease in serum glucose concentration on day 14 (0.6 ± 5.3) compared to control (1.1± 10.2). Similarly, rats in group C treated with 800 mg/kg of the extract showed significant ($P<0.05$) decrease in serum glucose concentration on day 21 (0.5 ± 4.5) compared to pre-treatment value (0.9 ± 10.8) and control (1.6 ± 9.1). These changes showed fluctuations in the withdrawal period in all the treatment groups.

Table 1. Effect of prolonged administration of crude extract of wild *Ganoderma lucidum*on serum glucose concentration (mmol/L) in albino rats

Groups	Dose of extract (mg/kg)	Before treatment	Treatment (days) Mean (± SD) value of serum glucose concentration (g/dl)			Post treatment (days)	
		0	7	14	21***	28	35
A	200	0.6±4.3	0.8±3.4	0.4±7.1[a]	0.4±2.1[a]	0.6±11.2	0.7±14.1
B	400	0.6±6.1	0.5±6.1	0.6±5.3[b]	0.5±0.8	0.5±8.5	0.6±5.1
C	800	0.9±10.8[c]	0.5±12.0	0.7±6.5	0.5±4.5[a]	0.8±4.5[b]	0.6±5.1
D	Water	0.5±8.1	0.5±5.0	1.1±10.2	1.6±9.1	0.4±9.9	0.8±12.1

*** = Termination of treatment.

[a]= Significant (P<0.05) decrease compared to control and value before treatment

[b]= Significant (P<0.05) decrease compared to control

[c]= Significant (P<0.05) increase compared to control.

DISCUSSION

Remarkable non-dose dependent decrease in serum glucose concentrations were observed in all the treatment groups at different weeks of treatment compared to pre-treatment and control. This may be due to increase in glucose uptake as reported by Jung *et al.* (2006) or due to inhibition of alpha-glycosidase enzyme [7-9]. It can also be due to increase in insulin secretion by the pancreatic cells stimulated by the extract that enables efficient utilization of ingested and absorbed glucose (Ni and Fournier, 2010). The decrease in glucose concentration suggest the potential use of *Ganoderma lucidum* methanolic extract as an anti-diabetic agent as reported by Zhang and Liu (2004) [6]. Chromium is reported to modulate the insulin activity [6], and this element was reported to be present in *Ganoderma lucidum* extracts. Shamaki *et al.* this may have significantly regulated the amount of glucose level in the serum. Glucose levels increase within the observed withdrawal period when extract administration was withdrawn, further indicating the hypoglycemic effect of the methanolic extract of *Ganoderma lucidum.*

CONCLUSION

Crude methanolic soluble fraction of the wild *Ganoderma lucidum* extract can be used in the management of disease such as diabetes mellitus

RECOMMENDATION

The methanolic fractions of the wild *Ganoderma sp.* extract should be used at 200-400 mg/kg for 7-14 days in the management of insulin dependent diabetes mellitus

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