

Full Length Research Paper

Effects of aqueous extract of *Ganoderma lucidum* on blood glucose levels of normoglycemic and alloxan-induced diabetic wistar rats

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Accepted 02 November, 2018

Ganoderma lucidum mushroom has long been used as a potent medicinal plant in the far East, but its ethnomedicinal importance in Nigeria is yet to be fully realized. The effects of the aqueous extract on blood glucose levels of alloxan induced and normal Wistar rats have been investigated. Three doses of the extract (250, 500 and 1000 mg/Kg) were administered intraperitoneally. The dose of 250 mg/Kg of the extract did not significantly alter the blood glucose levels of both normal and alloxan induced diabetic Wistar rats. However, the doses of 500 and 1000 mg/Kg of the extract did significantly ($p < 0.05$) decrease the blood glucose levels of alloxan diabetic Wistar rats at 4, 8 and 24 h. In normal rats, the dose of 1000 mg/Kg of the extract significantly ($p < 0.05$) decrease the blood glucose levels at 8 and 24 h only. In conclusion, the dose of 1000 mg/Kg of the extract has shown both significant ($p < 0.05$) hypoglycemic and anti-hyperglycemic effects in Wistar rats.

Key words: *Ganoderma lucidum*, alloxan diabetes, blood glucose.

INTRODUCTION

Diabetes mellitus (DM) is a major health problem all over the world. Globally, the number of people that has been diagnosed with diabetes has exploded in the past two decades. In 2000, 151 million people in the world were diabetic. With the current rate of increase (6% per annum), it has been projected that 221 million people will be diabetic in 2010 and 324 million by 2025 (Zimmet et al., 2001). Several approaches were made to reduce the hyperglycemia, the hallmark of diabetes mellitus, with treatments such as sulfonylureas, which stimulates pancreatic islet cells to secrete insulin; metformin, which acts to reduce hepatic glucose production; α -glucosidase inhibitors, which interfere with glucose adsorption and insulin itself, which suppresses glucose production and augments glucose utilization (Moller, 2001). The growing public interest and awareness of natural medicines have led the pharmaceutical industry and academic researchers to pay more attention to medicinal plants (Day, 1998). The apparent reversal of trend from western to herbal medi-

cine is partly due to the fact that synthetic drugs have always shown adverse reactions and other undesirable side effects. This has led to the belief that natural products are safer because they are more harmonious with biological systems. In addition, the cost of administering modern antidiabetic drugs is beyond the reach of people in the low income group and those living in the rural areas. In Nigeria, hundreds of plants are used traditionally for the management of diabetes mellitus. To date, however, only a few of these medicinal plants have received scientific scrutiny, despite the fact that the World Health Organization has recommended that medical and scientific examinations of such plants should be undertaken (WHO, 1980).

Mushrooms have a notable place in the folklore throughout the world and in the traditions of many cultures (Chang and Bushwell, 1996). The family of *Ganodermataceae* consists of a large group of tree fungi of the class *Polyporaceae*, specifically the genus *Ganoderma* and other related genera. *Ganoderma* fungi are mainly found in tropical and subtropical areas; the typical species is *Ganoderma lucidum* (Fr.) Karst. It is called Ling Zhi or Reishi (Chinese), Mannentake (Japanese) and Tuwon

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biri (Hausa). It is seasonal and can be found growing alone or in groups on decaying hardwood logs and stumps. At first, the caps are irregularly knobby or elongated, but by maturity more or less fan-shaped; with a shiny, varnished surface often roughly arranged into lumpy "zones"; red to reddish brown when mature; when young often with zones of bright yellow and white toward the margin. The stem is sometimes absent, but more commonly present; 3 - 14 cm long; up to 3 cm thick; twisted; equal or irregular; varnished and colored like the cap; often distinctively angled away from one side of the cap. The under side is cream colored and porous. *G. lucidum* is commonly known as a medicinally-potent mushroom. It has been widely used in China and other oriental countries for hundreds of years for the treatment of various diseases, including cancer. This mushroom is reported to have various biological activities, such as anti-tumor, antibacterial, and antiviral activities (Yoon et al., 1994; Wang et al., 1997; El-Mekkawy et al., 1998; Eo et al., 2000). It was also reported to have an anti-inflammatory and liver protective effect in rats (Lin et al., 1993; Lin et al., 1995). The aim of this research work is to investigate the effects of aqueous extract of *G. lucidum* on blood glucose levels of normoglycemic and alloxan-induced diabetic Wistar rats. This would help in contributing towards the ethnobotanical uses of mushrooms found in Nigeria.

MATERIALS AND METHODS

Plant material

Fruiting bodies of *G. lucidum* were collected within Main campus, Ahmadu Bello University, Zaria in the month July 2006. The plant material was identified by Dr. P. A. Wuyep of Biological Science Department A.B.U., Zaria where a voucher specimen (No.BSTCC 005) has been deposited at the herbarium unit.

Extract preparation

The fruiting bodies were dried under the shade and ground into powder. The powder (500 g) was macerated in 2.5L of distilled water at room temperature for 24 h. It was then filtered using a filter paper (Whatmann size no.1) and the filtrate evaporated to dryness in water bath at 60°C. A brownish residue weighing 25.5 g (yield of 5.1% w/w) was obtained. This was kept in air tight bottles in a refrigerator until used.

Chemicals used

All chemicals and drugs were obtained commercially and were of analytical grade.

Acute toxicity study

The method of Lorke (1980) was adopted and a total of 24 rats weighing 120 - 145 g each were used for this study. The animals were fasted for 12 h before the study, but were allowed water *ad libitum*. In the initial phase, four groups (n = 3) were given normal saline as control group and 100, 1000 and 10,000 mg/Kg of the ex-

tract intraperitoneally (i.p) for the remaining three groups respectively. They were then observed for 24 h for signs of toxicity or deaths. In the final phase, another four groups (n = 3) were given normal saline, 2000, 4000 and 8000 mg/Kg of the extract i.p for the remaining groups respectively and were observed for 24 h for signs of toxicity or deaths. The median lethal dose (LD₅₀) was calculated from the final phase.

Animals and induction of diabetes mellitus

Fifty Wistar rats of both sexes weighing 120 - 180 g were used for the study of the effects of aqueous extract of *G. lucidum* on the blood glucose levels of the animals. They were kept in standard cages at 25°C and 12 h light/dark condition in the animal room of the Department of Human Physiology, ABU, Zaria. They were fed on commercial rats' feeds and were given water *ad libitum*. The animals were fasted from feeds for 12 h before the commencement of each experiment, but were allowed water *ad libitum*. The rats assigned to the diabetic groups were injected with a freshly prepared alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight intraperitoneally. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, the rats were treated with 20% glucose solution intraperitoneally after 6 h (Stanley et al., 2001). The rats were kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia (Stanley et al., 1997). After a period of two weeks the rats with a blood glucose levels greater than 150 mg/dl were considered diabetic and used for this research work (Stanley et al., 2001).

Experimental design

The alloxan-induced diabetic Wistar rats were randomly assigned into five groups (1 - 5) of five rats (n = 5) each as follows, namely: Group 1- Received 250 mg/kg body weight of the aqueous extract i.p; Group 2-Received 500 mg/kg body weight of the aqueous extract i.p; Group 3-Received 1000 mg/kg body weight of the aqueous extract i.p; Group 4-Received Biphasic Isophane Insulin 6 i.u/kg i.p (Stanley et al., 2001); Group 5-Received with normal saline i.p

The normoglycemic Wistar rats were also randomly grouped into five (6 - 10) with five rats (n = 5) in each group as follows, namely: Group 6-Received with normal saline i.p; Group 7-Received 250 mg/kg body weight of the extract i.p; Group 8-Received 500 mg/kg body weight of the extract i.p; Group 9-Received 1000mg/kg body weight of the extract i.p; Group10-Received Biphasic Isophane Insulin 6 i.u/kg i.p (Stanley et al., 2001)

Determination of blood glucose levels

All blood samples were collected by cutting the tail- tip of the rats. Blood samples for blood glucose determination were collected from the tail at intervals of 0, 2, 4, 8 and 24 h. Determination of the blood glucose level was done by the glucose-oxidase principle (Beach and Turner, 1958) using the ONE TOUCH Basic (Lifescan, Milpitas, CA) instrument and results were reported as mg/dl (Rheney and Kirk, 2000).

Statistical analysis

Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett's method. Values of p<0.05 or less were taken as significant. The alloxan-induced dia-

Table 1. Effects of aqueous extract of *Ganoderma lucidum* on blood glucose levels of Alloxan-induced diabetic Wistar rats.

Treatment	Blood glucose levels(mg/dl)				
	0 hour	2 hours	4 hours	8 hours	24 hours
Control(N/Saline)	270.6±30.65	241.1±30.66	324.2±29.54	353.4±29.25	330.0±23.59
Insulin	279.4±36.08	308.6±42.20 ^{ns}	279.8±33.82 ^{ns}	297.8±37.28 ^{ns}	349.8±30.57 ^{ns}
250mg/kg	286.8±33.98	337.6±54.78 ^{ns}	364.8±17.96 ^{ns}	391.8±22.82 ^{ns}	431.6±31.30 ^{ns}
500mg/kg	240.0±53.49	259.2±34.66 ^{ns}	125.4±42.65 ^a	72.0±23.67 ^a	154.8±53.74 ^a
1000mg/kg	278.8±42.37	270.8±27.49 ^{ns}	162.0±58.92 ^a	73.8±27.71 ^a	126.8±47.10 ^a

^a=P<0.05=Significant, ^{ns}= not significant n = 5

diabetic groups and normoglycemic groups were analyzed separately for statistical significance.

RESULTS

Acute toxicity study

Signs of toxicity were first noticed after 4 - 5 h of aqueous extract administration. There were decreased locomotor activity and sensitivity to touch and pain. Also there was decreased feed intake, tachypnoea and prostration after 8 - 12 h of aqueous extract administration. In the final phase, the mortality rates for 2000, 4000 and 8000 mg/Kg of the extract were 0, 66.6 and 100% respectively. The LD₅₀ was calculated as 3500 mg/kg by log-probit method.

Blood glucose levels of alloxan-induced diabetic Wistar rats

Table 1 showed the results of the effects of three doses (250, 500 and 1000 mg/Kg) of the aqueous extract of *G. lucidum*, insulin and control groups in alloxan diabetic Wistar rats. The doses of insulin and 250 mg/Kg of the extract did not show any significant change in the blood glucose levels when compared to untreated control. However, the doses of 500 and 1000 mg/Kg of the extract showed a significant (p<0.5) decrease in the blood glucose levels after 4, 8 and 24 h.

Blood glucose levels of normoglycemic Wistar rats

Table 2 showed the results of the effects of three doses (250, 500 and 1000 mg/Kg) of the aqueous extract of *G. lucidum*, insulin and control groups in normal Wistar rats. The dose of insulin showed a significant (p<0.05) decrease of blood glucose levels at 2 and 8 h. The extract did not significantly alter the blood glucose levels at a dose of 250 mg/Kg body weight. The dose of 500 mg/Kg did significantly (p<0.05) decrease the blood glucose levels after 8 h of treatment only. However, the dose of

1000 mg/Kg did significantly (p<0.05) decrease the blood glucose levels after 8 and 24 h of treatment.

DISCUSSION AND CONCLUSION

Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus. It induces diabetes by partial destruction of the β -cells of Islets of Langerhan's (Abdel-Barry et al., 1997). This results in decreased insulin levels and hyperglycemia leading to type 1 diabetes mellitus. However, animal models of diabetes differ significantly from each other and none can be taken, without reservation, to reproduce the essentials of human diabetes (Bell and Hyde, 1983).

G. lucidum polysaccharides have been shown to have hypoglycemic potentials in normal and glucose loaded mice and rats by increasing the plasma insulin levels, but did not affect the insulin binding to isolated adiposities (Hikino et al., 1985, 1989). Also, Hikino and Mizuno (1989) in a similar study reported the hypoglycemic activity of fifteen heteroglycans fractions of *G. lucidum* administered intraperitoneally at a dose of 100 mg/kg in rats. After 7 h of administration, all but two showed significant hypoglycemic activity and all but four showed activity after 24 h of administration. In another study, Ki-mura et al. (1988) reported the anti-hyperglycemic effects of *G. lucidum* in rats with glucose induced hyperglycemia. Furthermore, Zhang and Lin (2004) reported the hypoglycemic effects of *G. lucidum* polysaccharides in normal fasted rats.

Thus, the results of this study of the aqueous crude extract of *G. lucidum* on the blood glucose levels of normal and alloxan induced diabetic Wistar rats were in consonance with the findings of earlier researchers. The dose of 250 mg/Kg of the extract did not significantly alter the blood glucose levels of both normal and alloxan induced diabetic Wistar rats. However, the doses of 500 and 1000 mg/Kg of the extract did significantly (p<0.05) decrease the blood glucose levels of alloxan diabetic Wistar rats at 4, 8 and 24 h. In normal rats, the dose of 1000 mg/Kg of the extract significantly (p<0.05) decrease the blood glucose levels at 8 and 24 h only. In conclusion, the dose of 1000 mg/Kg of the extract has shown both significant (p<

Table 2. Effects of aqueous extract of *Ganoderma lucidum* on blood glucose levels of Normoglycemic Wistar rats.

Treatment	Blood glucose levels(mg/dl)				
	0 hour	2 hours	4 hours	8 hours	24 hours
Control (N/Saline)	66.2±4.26	76.4±6.78	84.4±7.93	90.8±10.61	77.6±6.61
Insulin	72.0±3.97	49.0±2.57 ^a	58.2±12.63 ^{ns}	65.8±6.97 ^a	88.6±5.21 ^{ns}
250mg/kg	66.4±3.25	75.0±5.50 ^{ns}	71.2±8.67 ^{ns}	69.0±5.91 ^{ns}	79.8±7.12 ^{ns}
500mg/kg	65.8±1.65	104.8±10.09 ^{ns}	57.4±8.61 ^{ns}	52.6±3.91 ^a	76.2±5.08 ^{ns}
1000mg/kg	65.0±1.09	107.4±12.52 ^{ns}	54.4±6.72 ^{ns}	39.6±2.25 ^a	48.6±6.65 ^a

^a=P<0.05 = Significant, ^{ns}= not significant n = 5.

0.05) hypoglycemic and anti-hyperglycemic effects in Wistar rats.

ACKNOWLEDGEMENTS

The authors wish to thank Mallam Bala Mohammed, a technical staff of the Department of Human Physiology, ABU, Zaria for the care of the experimental animals throughout the period of this research work.

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