Evaluation of the hypoglycemic effect of aqueous extract of *Ganoderma lucidum* on STZ-induced diabetic wistar rats

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ABSTRACT

This study was undertaken to investigate the potential hypoglycemic effect of aqueous extract of *Ganoderma lucidum* in normal and streptozotocin (STZ)-induced hyperglycemic rats. The study comprises three groups of normal rats administered 0, 100, and 200 mg/kg body weight and another three groups of diabetic rats administered 0, 100, and 200 mg/kg body weight of extract. The extract was given by gavage once daily for four weeks. Body weight and feed intake were monitored weekly while serum glucose, insulin, hemoglobin glycosylation level, and lipid profile were measured at baseline (week 0), two weeks and four weeks after treatment. *G. lucidum* dose dependently decreased food intake, body weight, serum glucose in both normal and STZ-diabetic rats. On the other hand, the extract increased serum insulin level in both normal and STZ-diabetic rats in a dose-dependent fashion. In addition, it also improved serum lipid profile in both normal and diabetic animals. These results suggest that aqueous extract of *G. lucidum* protect against STZ-induced diabetic in rats.

Key words: Hyperglycemia, *Ganoderma lucidum*, insulin, hemoglobin glycosylation, hypoglycemia.

INTRODUCTION

Diabetes mellitus is a chronic disease characterized by elevated plasma glucose concentration resulting from insulin insufficiency and/or insulin resistance [1]. Much of the increase incidence of diabetes worldwide occurs in developing countries with such cases as ageing, unhealthy diets, obesity and sedentary lifestyle, with malnutrition-related causes playing a pivotal role.
Plants have always been utilized as sources of drugs and many of the currently available drugs have been directly or indirectly obtained from plant sources. In accordance with the recommendations of the WHO Committee on diabetes mellitus, it is important to investigate the hypoglycemic actions from plants which were originally used in traditional medicine [3].

*Ganoderma lucidum* is a basidiomycetes, lamellaless fungus belonging to the family of Polyporaceae. Its fruiting body has long been used in China, Japan, and Korea as a traditional or folk medicine for the treatment of wide varieties of diseases. Recent studies on this mushroom have demonstrated many interesting biological activities, including antitumour [4], hypotensive [5], cytotoxicity [6], anticomplementary [7], antimicrobial [8], hepatoprotective [9], hypolipidemic [5], and anti-inflammatory [10] effects. Qualitative and quantitative differences in the chemical composition of *G. lucidum* extract depending on the strain, origin, extracting process and cultivation conditions have been reported in these studies. Several studies from China, Australia, and Japan have already been carried out on the hypoglycemic effect of the extracts of *G. lucidum* fruiting body. However, report on the anti-diabetic property of *G. lucidum* extract from Nigeria is scarce. Hence the need to evaluate the anti-diabetic property of aqueous extract of *G. lucidum* from Nigeria in this study is warranted.

**MATERIALS AND METHODS**

**Plant material:** Fruiting bodies of *G. lucidum* were collected from around Iwaro Oka Akoko in the month of June 2009. The plant material was identified and authenticated by Dr. Obembe of Plant Science Department, Adekunle Ajasin University, Akungba.

**Extract preparation:** The fruiting bodies were dried under shade and ground to powder using mechanical grinder. About 500 g of the powder was macerated in 2.5 L distilled water at room temperature for 24 h. It was then filtered using Whatmann filter paper (number 1) and filtrate evaporated to dryness in water bath at 60°C. A brownish residue about 46.3 g (9.26% yields) was obtained and kept in airtight container at 40°C until used.

**Experimental animals:** 60 male Wistar rats weighing 85 – 100 g obtained from the animal laboratory of the Department of Biochemistry, University of Ilorin, Ilorin were used for the study. The animals were housed individually in stainless steel cages with raised wire floor at 25°C and 12 h light/darkness condition. Maintenance and treatment of animals were in accordance with the principles of the “Guide for care and use of laboratory animals in research and teaching” prepared by the National Academy of Sciences and published by the National Institute of Health (NIH) publication 86 – 23 revised in 1985. They were fed commercial rat’s feed (Bendel Flour Mill Ltd, Ewu) and water *ad libitum* for the initial period of two weeks of acclimatization and throughout the experiment.

**Acute toxicity study:** 25 rats selected from the above animals were used to evaluate the acute toxicity of the extract. The animals were divided into five groups (n = 5) after been fasted overnight but allowed access to water *ad libitum*. Animals in each group were given different dose of the extract by gavage as follow: 0, 50, 100, 200, 400 mg/kg body weight respectively. Animals receiving 0 mg/kg serving as control were given normal saline. Animals in each group were then observed for 24 h for signs of toxicity or death. The number of death(s) in each group...
was/were recorded. The LD<sub>50</sub> of the extract was estimated from the graph of percentage (%) mortality (converted to probit) against log<sup>dose</sup> of the extract, probit 5 being 50%.

**Experimental induction of diabetes:** After two weeks of acclimatization animals were fasted for 12 h but allowed access to water ad libitum before an intraperitoneal (i.p.) injection of STZ (Sigma, UK, 65 mg/kg body weight, dissolved in citrate buffer at pH 4.5) [11]. Two days after the STZ treatment, the rats were considered to be diabetic when the non-fasting blood glucose concentrations were higher than 300 mg/dl. The diabetic state was further confirmed by the positive response to glucose in the urine (test strips; Glucotest, Germany). Thereafter, the animals were used as an insulin-dependent diabetes mellitus (IDDM) model.

**Experimental design:** Thirty rats weighing 95 – 115 g divided into six groups consisting of three groups (n = 5) of normal rats designated: Normal control, Normal test A and Normal test B respectively; and three groups (n = 5) of diabetic rats designated: Diabetic control, Diabetic test A and Diabetic test B respectively. Animals in the two control groups received 10 ml/kg normal saline, animals in test A group (both normal and diabetic) were given 100 mg/ kg extract while the two test B groups (normal and diabetic) received 200 mg/kg extract. The treatment was administered by gavage once daily. Before the commencement of the treatment animals in each group were fasted overnight but allowed accesses to water ad libitum. Rats in each group were fed commercial rat’s feed and water ad libitum throughout the treatment period. Body weight of the animals and feed intake per group were monitored weekly.

**Serum preparation:** Blood samples collected for the vein in the tail of animals in each group at weeks 0, 2 and 4 into plain tubes.

**Biochemical analysis:** Serum glucose and insulin level were determined using a glucose oxidase kit glucose B-test, (Wako Chemicals, Japan) [12] and by <sup>125</sup>I-radioimmunoassay (Coat-A-Count Insulin kit, DPC Co., U. S. A.) [13], respectively. Hemoglobin glycosylation level was measured according to the method of Nayak and Pattabiraman [14]. Serum total cholesterol (TC), triacylglycerol (TAG) and HDL cholesterol (HDL-C) were measured by enzymatic test kits (Randox Laboratory Ltd, UK). LDL cholesterol (LDL-C) was calculated using Friedewald equation [15].

**Statistical analysis:** Results are expressed as mean ± SEM. Group means were compared by a one way analysis of variance and by Duncan’s Multiple Range Test. P < 0.05 was considered significant.

**RESULTS**

**Food intake:** The effect produced by the administration of aqueous extract of *G. lucidum* on weekly feed intake of normal and STZ-diabetic rats is shown in figure 1. Weekly feed intake was significantly reduced (p<0.05) by the extract administration in both normal and diabetic rats compared to control. The effect on feed intake was not dose dependent as there was no significant (p>0.05) difference between the groups administered 100 and 200 mg/kg of the extract.
Body weight gain: The effect of the extract on weekly body weight gain of both normal and STZ-diabetic rats is shown in figure 2. Significant decreases (p<0.01) in body weight were observed in the body weight of normal rats administered 100 and 200 mg/kg of the extract compared to normal control. Similarly, body weight was significantly reduced (p<0.01) in STZ-diabetic rats administered 100 and 200 mg/kg extract compared to diabetic control. No significant difference was observed in the body weights of rats in diabetic tests A and B.

Effect on serum glucose level: Serum glucose concentration was significantly (p<0.05) reduced in normal rats given 100 and 200 mg/kg aqueous extract of *G. lucidum* compared to normal control (fig. 3). The extract also produced significant (p<0.01) decrease in glucose level of diabetic rats. The decrease was observed to be dose dependent as greater decrease was observed.
in diabetic rats receiving 200 mg/kg extract compared to those administered 100 mg/kg extract (fig. 3).

\[\text{Figure 3: Effect on serum glucose concentration (mg/dl)}\]

**Effect on serum insulin level**: The effect observed on serum insulin level by the administration of the extract is presented in figure 4. The results shown that aqueous extract of *G. lucidum* at 100 and 200 mg/kg body weight caused significant (p < 0.05 and 0.01 respectively) stimulation of insulin release into the serum of normal and STZ-induced diabetic rats.

\[\text{Figure 4: Effect on serum insulin level (mIU/L)}\]

**Effect on hemoglobin glycosylation**: Administration of aqueous extract of *G. lucidum* at 100 mg/kg body weight did not show significant change (p>0.05) in level of glycosylation of serum hemoglobin (expressed as % total hemoglobin) in normal rats within the first two weeks of treatment but significantly (p<0.05) reduced serum hemoglobin glycosylation rate after four
weeks of administration (fig 5). On the other hand level of serum hemoglobin glycosylation was significantly reduced (p<0.01) at two and four weeks of G. lucidum administration (both at 100 and 200 mg/kg body weight) in STZ-diabetic rats compared to STZ-diabetic control rats.

![Figure 5: Effect of serum Hb glycosylation (% total Hb)](image)

### Table 1: Effect on lipid parameters

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Lipid parameters</th>
<th>Normal control</th>
<th>Normal test A</th>
<th>Normal test B</th>
<th>Diabetic control</th>
<th>Diabetic test A</th>
<th>Diabetic test B</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>TAG</td>
<td>101.3±17.2</td>
<td>103.5 ±7.8</td>
<td>101.0 ± 12.5</td>
<td>143.5±15.5</td>
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<td></td>
<td>TC</td>
<td>74.7±3.5</td>
<td>74.3±5.5</td>
<td>74.7±2.6</td>
<td>112.1±8.5</td>
<td>109.6±8.3</td>
<td>111.9±7.5</td>
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<td></td>
<td>VLDL-C</td>
<td>17.2±2.1</td>
<td>16.3±1.5</td>
<td>16.4±1.4</td>
<td>25.8±2.4</td>
<td>25.2±2.2</td>
<td>25.7±3.1</td>
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<tr>
<td></td>
<td>LDL-C</td>
<td>50.8±2.5</td>
<td>53.5±2.3</td>
<td>51.5±1.5</td>
<td>78.5±3.6</td>
<td>76.7±3.3</td>
<td>78.3±4.2</td>
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<td>HDL-C</td>
<td>6.7±0.4</td>
<td>4.8 ± 0.4</td>
<td>6.5 ± 0.5</td>
<td>7.8±1.2</td>
<td>7.7±1.0</td>
<td>7.8±1.5</td>
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<tr>
<td>2</td>
<td>TAG</td>
<td>103.8±6.5</td>
<td>101.5±12.3</td>
<td>98.7±9.3</td>
<td>148.6±12.8</td>
<td>136.5±17.2</td>
<td>127.3±15.0</td>
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<td>TC</td>
<td>75.3±3.2</td>
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<td>VLDL-C</td>
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<td>LDL-C</td>
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<td>HDL-C</td>
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<td>4</td>
<td>TAG</td>
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<td>36.4±3.2</td>
<td>29.8±3.5</td>
<td>26.0±2.5</td>
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<td>LDL-C</td>
<td>54.1±4.0</td>
<td>41.6±2.5</td>
<td>38.3±3.1</td>
<td>110.8±12.5</td>
<td>93.4±5.7</td>
<td>84.0±3.4</td>
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<tr>
<td></td>
<td>HDL-C</td>
<td>7.1±1.2</td>
<td>7.0±1.2</td>
<td>7.2±1.2</td>
<td>11.1±2.0</td>
<td>12.2±2.2</td>
<td>13.6±2.1</td>
</tr>
</tbody>
</table>

*Significantly different from normal control. **Significantly different from diabetic control.

**Note:** TAG, triacyl glycerol; TC, total cholesterol; VLDL-C, very-low density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

**Effect on serum lipid profile:** The effect of the extract on serum lipid profile of normal and STZ-diabetic rats is shown in Table 1. From the results it is observed that the extract produced a significant decrease in serum TAG, TC, VLDL-C and LDL-C in normal rats and STZ-diabetic rats.
rats. In addition, serum HDL-C was significantly increased in STZ-diabetic rats given 200 mg/kg of the extract at the end of the fourth week.

**DISCUSSION**

For several decades, the β-cell specific toxin streptozotocin (STZ), an analogue of GlcNAc, has been used to create animal models of diabetes. STZ action in β-cell is accompanied by alterations in blood insulin and glucose concentrations. Two hours after STZ injection, the hyperglycemia is observed with a concomitant drop in blood insulin.

About six hours later, hyperglycemia occurs with high levels of blood insulin. Finally, hyperglycemia develops and blood insulin levels decrease [16]. The changes in blood glucose and insulin concentrations reflect abnormalities in β-cell function. STZ thus impairs glucose oxidation [17] and decreases insulin biosynthesis and secretion [18,19].

Diabetes mellitus is a complex metabolic disease caused by impairment of insulin-signaling pathways, and the defect usually results from pancreatic β-cell deficiency and/or a deficiency of insulin [20]. Diabetes mellitus is characterized by many chronic complications such as vascular disease, retinopathy, neuropathy, kidney disease and heart disease. Cardiovascular disease is a major cause of death in diabetic patients. Diabetes mellitus is associated with profound alteration in serum lipid and lipoprotein profiles with increased risk of coronary heart disease (CHD) [21]. Hyperlipidemia is a recognized complication of diabetes mellitus characterized by elevated levels of cholesterol, triacyl glycerol and phospholipids, and changes in lipoprotein composition [22].

Due to their high content of fibre and protein and low fat content, extract of edible mushroom have been considered to be ideal foods for dietetic prevention of hyperglycemia [23]. Extracts of several medicinal mushrooms, including *Tremell aurantia*, ‘*Cordyceps sinensis*’, *Ganoderma lucidum* and *Auricula-judae* have been reported to demonstrate hypoglycemic activity [24, 25]. The blood glucose and triglyceride (TG) lowering effect of water soluble extracts from *Lentinus edodes*, *Pleurotus ostreatus* and *Phellinus linteus* in the STZ-induced diabetic model have been clearly demonstrated [26]. Such reports strongly suggest that these mushrooms have potential preventive and therapeutic action in diabetes mellitus (type I and II).

*G. lucidum* (Fr.) Karst, a fungus (*Polyporaceae*) used in traditional Chinese medicine, has attracted great attention recently. It produces polysaccharides with antitumour and hypoglycemic activities. Zhang *et al*. [27] have reported that polysaccharides from *G. lucidum* showed strong hypoglycemic properties. Report from their study showed that *G. lucidum* polysaccharides (Gl-PS) at 100 mg/kg caused increased of serum insulin level at 60 min and decreased serum glucose levels. Concluding on the report of their study they hypothesized that the hypoglycemic action of Gl-PS may be due to its ability to rapidly stimulate liver glycogen decomposition in a short time thus reduced the glycogen content in liver. Results obtained from this study showed that aqueous extract of *G. lucidum* decreased feed intake in both normal and STZ-induced diabetic mice. This effect may result from the fact that the extract is bitter in taste due to its high content of triterpenes. This is coupled with the fact that it was administered intragastrically. This phenomenon may also account for the observed
decrease in body weight of the animals. The significant reduction in feed intake in the animals signifies a significant decrease in energy intake thus reduced body weight.

Results of the present study also show that *G. lucidum* extract decrease serum glucose concentration, and increase serum insulin levels. This is consistent with reports of earlier investigators. *G. lucidum* has been found to be effective in reducing blood glucose level. *Ganoderan B*, one of the major bioactive components of *G. lucidum* was considered to enhance glucose utilization because it increased the plasma insulin level in normal and glucose loaded mice [28]. It is thus believed that the hypoglycemic activity of *G. lucidum* is due to an increase in serum insulin level and an acceleration of glucose metabolism occurring not only in the peripheral tissues but also in the liver.

Worthy of note is the effect of *G. lucidum* extract on serum hemoglobin glycosylation. Glycation of serum and tissue membrane protein is a free radical-mediated phenomenon. Reports from recent experimental studies have proved that the main reason for STZ-induced β-cell death is alkylation of DNA [29, 30]. STZ has also been reported to be a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells, NO has also been proposed to contribute to STZ-induced DNA damage [31]. The observed increase in serum hemoglobin glycosylation rate in STZ-induced rats suggests an increased rate of alkylation and free radical formation. The effect of *G. lucidum* extract in bringing about a significant reduction in rate of serum hemoglobin glycosylation may suggest that the extract contain some antioxidant principle.

In addition, results obtained in this study indicate that aqueous extract of *G. lucidum* exhibit hypolipidemic action STZ-diabetic rats. The extract shows no significant effect on serum lipid profile in normal rats (Table 1). However, significant reductions were observed in serum total cholesterol (TC), triacyl glycerol (TAG), and LDL-C and significant increase in HDL-C in STZ-diabetic rats. This observation is in agreement with the reports of Zhang et al. [4] who had earlier reported that exo-biopolymer obtained from submerged mycelial culture of *G. lucidum* lowered the plasma TC, TAG, LDL-C and increased plasma HDL-C in STZ-diabetic rats. It could thus be concluded that aqueous extract of *G. lucidum* is hypolipidemic in diabetic rats. This fact could also provide evidence for the weight reducing action of the extract in diabetic animals as observed in this study. Increase in HDL-C and reduction in LDL-C, TC and TAG observed in this study could be considered beneficial in the long-term prognosis of diabetic subjects. The ability of the extract to re-distribute serum lipoprotein in favour of good cholesterol (HDL-C) may suggest its possible effect in stimulating reverse cholesterol transport to the liver.

In conclusion, treatment with aqueous extract of *G. lucidum* was found to produce anti-hyperglycemic effect in STZ-induced hyperglycemic rats. One mechanism may be through inhibition of serum hemoglobin glycosylation thus protecting against STZ-induced β-cell death as a result of alkylation of DNA.

REFERENCES


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