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Antidiabetic Properties of the Tiger's Milk Medicinal Mushroom, *Lignosus rhinocerotis* (Agaricomycetes), in Streptozotocin-Induced Diabetic Rats

Kar Lin Nyam,[1,*] Cheng Fhai Chow,[1] Chon Seng Tan,[2] & Szu Ting Ng[3]

[1]Department of Food Science with Nutrition, Faculty of Applied Sciences, UCSI University, Jalan Menara Gading, UCSI Heights (Taman Connaught), Cheras, Kuala Lumpur, Malaysia; [2]Malaysian Agricultural Research and Development Institute, Selangor, Malaysia; [3]Ligno Biotech Sdn. Bhd., Balakong Jaya, Selangor, Malaysia

*Address all correspondenece to: Kar Lin Nyam, Department of Food Science with Nutrition, Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights (Taman Connaught), Cheras, 56000 Kuala Lumpur, Malaysia; Tel.: +603-91018880; Fax: +603-91022614; nyamkl@ucsiuniversity.edu.my

ABSTRACT: Diabetes mellitus is a major cause of morbidity and mortality worldwide. Although scientific evidence supporting its therapeutic efficacy is lacking, the use of the tiger's milk mushroom (TGM; *Lignosus rhinocerotis*), which is native to tropical areas such as Malaysia, Indonesia, and the Philippines, has been found to contain a very large amount of potential antioxidants. In this study, rats were weighed and then intravenously injected with 35 mg/kg streptozotocin (STZ). Rats were left for 1 week before blood glucose concentrations were measured to determine the onset of diabetes before the next procedure was conducted. Rats with blood glucose exceeding 7.0 mmol/L were considered diabetic and were included in the experiment. All groups were fed their respective treatments twice daily for 2 months throughout the experiment. Antidiabetic and antioxidant properties of freeze-dried TGM powder, such as reduced glutathione (GSH), superoxide dismutase (SOD), lipid peroxidation (LPO), and catalase (CAT) activities, were investigated in liver samples. The biological compounds present in the freeze-dried TGM powder was found to exhibit antidiabetic properties by significantly reducing elevated blood glucose concentrations to a normal range (3.0–7.0 mmol/L) in Sprague-Dawley rats with streptozotocin-induced diabetes,and increasing the body weight of the rats. Freeze-dried TGM powder was also found to possess antioxidant activity by significantly increasing GSH, CAT, and SOD activities while reducing LPO ($P < 0.05$). THis study shows that freeze-dried TGM powder exhibits significant antidiabetic properties and may be a potential supplement in ameliorating diabetic complications.

KEYWORDS: antidiabetic, antioxidant, *Lignosus rhinocerotis*, medicinal mushrooms, oxidative stress

ABBRIEVIATIONS: ALP, alkaline phosphatase; **ALT**, alanine transaminase; **AST**, aspartate transaminase; **CAT**, catalase; **GSH**, reduced glutathione; **H_2O_2**, hydrogen peroxide; **K_2HPO_4**, dipotassium phosphate; **KH_2PO_4**, monopotassium phosphate; **LPO**, lipid peroxidation; **SOD**, superoxide dismutase; **STZ**, streptozotocin; **TCA**, trichloroacetic acid

I. INTRODUCTION

The tiger's milk medicinal mushroom, *Lignosus rhinocerotis* (Cooke) Ryvarden (= *Polyporus rhinoceros*, Polyporaceae, Agaricomyces), is a valuable medicinal mushroom found in Malaysia, southern China, Thailand, Indonesia, Philippines, Papua New Guinea, New Zealand, and Australia. *L. rhinocerotis* is one of the most important medicinal mushrooms in Southeast Asia and southern China. It is widely used by Asians and Chinese as a general tonic and to treat diseases and conditions such as asthma, fever, breast cancer, and food poisoning. Few studies have investigated the various properties of the mushroom, such as its biological and pharmacological properties, because of its limited supply, as the mushrooms were only available in the jungle.[1] *L. rhinocerotis* was successfully cultivated commercially using readily available lignocellulosic agroresidues and spent brewery yeasts as the nitrogen source. The successful domestication of the mushroom enabled more research regarding its properties.[2]

A study of the chemical composition of *L. rhinocerotis* found that the fruiting body and sclerotium

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contained large amounts of polysaccharides, such as β-glucans, and dietary fiber, and small amounts of fat. The mushroom also contained moderate amounts of all essential amino acids except tryptophan. Large amounts of minerals such as potassium, phosphorus, and magnesium, and an appreciable amount of essential fatty acids, were detected in the mycelium.[2] In addition, the *L. rhinocerotis* fractions showed metal-chelating activity.[3]

This study was carried out to determine the antidiabetic properties of freeze-dried *L. rhinocerotis* powder orally administered to streptozotocin (STZ)-induced diabetic Sprague-Dawley rats.

II. MATERIALS AND METHODS

A. Mushroom Material

Freeze-dried *L. rhinocerotis* powder was obtained from Ligno Biotech Sdn. Bhd. (Selangor, Malaysia).

B. Sample Preparation

Freeze-dried *L. rhinocerotis* powder was weighed and mixed thoroughly with purified water before use.

C. Animals and Treatment

Male Sprague-Dawley rats weighing between 180 and 250 g were obtained from the institutional animal house of University Kebangsaan Malaysia. Rats were randomly divided into 6 groups, with 5 rats/group; groups were housed in plastic transparent cages under standard laboratory conditions. The rats were acclimatized for 14 days before the start of the experiments and were allowed free access to food and water throughout the study. Experimental procedures were carried out after approval from the by the ethics committee of the Faculty of Applied Sciences, UCSI University, Cheras, Kuala Lumpur (Ethical Clearance for Research [Proj-FAS-EC-14-019]). All the procedures were carried out in strict accordance with ethical committee guidelines for the care and use of laboratory animals.

D. Experimental Design

The rats were divided into 6 experimental groups (control, 3 samples, 1 negative control, and 1 positive control), with 5 rats in each group, then labeled and weighed before the weight of each rat was recorded. Rats in the control group received no treatment. The 3 sample groups were fed either 100, 250, or 500 mg/kg of *L. rhinocerotis* powder, respectively, after diabetes was induced. The negative control group was given distilled water after diabetes was induced. Metformin was used as the positive control drug.

E. STZ-Induced Diabetes

All rats were fasted overnight before diabetes was induced. After fasting, the rats were weighed and then 35 mg STZ/kg was injected intravenously. Weight was recorded for all rats. Rats were left for 1 week before blood glucose concentrations were measured to determine the onset of diabetes before the next procedure was conducted. Rats with blood glucose exceeding 7.0 mmol/L were considered diabetic and were included in the experiment. All groups were fed their respective treatments by oral gavage twice daily for 2 months throughout the experiment.

F. Blood Glucose Concentration and Weight

The blood glucose of the diabetic rats was measured every week using an Accu-Chek Performa blood glucose meter. Rats were fasted for 12 hours before blood glucose was measured. Blood samples were obtained by making a small cut at the tip of the rat's tail. The readings obtained were recorded.

G. Liver Tissue Extraction and Blood Collection

Rats were euthanized with diethyl ether before dissection. Blood samples were obtained via cardiac puncture and were stored in EDTA blood tubes. Liver tissues were extracted and gently washed

before they were pounded and homogenized with buffer solution. The tissue homogenates were then centrifuged to obtain a supernatant for further analyses.

H. Blood Analysis

Blood samples obtained from each group were sent to Gribbles Pathology (M) Sdn. Bhd. (Petaling Jaya, Malaysia) for full blood examination, liver function tests, lipid profiling, and renal function tests. The results obtained were used for statistical analysis.

I. Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was assayed according to the method described by Naskar et al.,[4] with slight modifications. Tissue homogenates with 20 mL of 0.025 M Tris-HCl buffer were centrifuged at 10,000 rpm at 4°C for 10 minutes. Absorbance of the supernatant obtained was read at 560 nm. The control solution was prepared by adding 1 mL sodium carbonate, 0.2 mL EDTA, and 0.4 mL hydroxylamine-HCl to the supernatant. The absorbance of the control was read at 560 nm after 2 minutes. The sample was prepared by adding 1 mL sodium carbonate, 0.4 mL nitro blue tetrazolium chloride, 0.2 mL EDTA, and 0.4 mL hydroxylamine-HCl. The absorbance was read at 560 nm after 2 minutes.

J. Catalase Activity

Catalase (CAT) activity was assayed according to the method described by Sinha,[5] which was based on the rate of hydrogen peroxide (H_2O_2) breakdown. Dichromate acetic acid was heated with H_2O_2 and reduced to perchromic acid, an unstable compound. The control was prepared by adding 4 mL of 0.01 M phosphate buffer (pH 8), which was prepared by adding 387 mL dipotassium phosphate (K_2HPO_4) and 613 mL monopotassium phosphate (KH_2PO_4) mixed with 1 L distilled water and 0.4 mL of 0.2 M H_2O_2, prepared via dilution by adding 20.40 mL of 30% (w/w) H_2O_2 solution to 1000 mL distilled water. The solution was left at room temperature for 3 minutes, then 2 mL dichromate acetic acid was added. The control solution was boiled for 10 minutes before absorbance was measured at 612 nm. The pH of the buffer was calibrated to 8 through the addition of 0.1 M sodium hydroxide.

Samples were prepared by homogenizing 1.8 g liver tissues with 6 mL of 0.15 M phosphate buffer. The buffer was prepared by adding 26.13 g K_2HPO_4 and 34.84 g KH_2PO_4 to 1000 mL distilled water. The homogenate were centrifuged at 10,000 rpm at 4°C for 10 minutes to obtain a supernatant. To 0.1 mL of the supernatant were added 4 mL of 0.01 M phosphate buffer (pH 8) and 0.4 mL of 0.2 M H_2O_2. The samples were left at room temperature for 3 minutes before 2 mL dichromate acetic acid was added; this was then boiled for 10 minutes. Absorbance of the sample was measured at 612 nm.

K. Lipid Peroxidation

The method described by Ohkawa et al.[6] was used to assay lipid peroxidation (LPO). Samples were prepared by centrifuging 2 g tissue homogenate and 20 mL Tris-HCl buffer at 10,000 rpm at 4°C for 10 minutes. The supernatant (1 mL) was then added to 1 mL of 0.9% saline solution, which was prepared by adding 9 g sodium chloride to 1000 mL distilled water and 2 mL of 10% trichloroacetic acid (TCA). The 10% TCA was prepared by adding 10 g TCA to 100 mL distilled water. The solution was then centrifuged again at 3000 rpm at 25°C for 10 minutes. The supernatant obtained (2 mL) was then added to 0.5 mL of 1% thiobarbituric acid prepared by adding 0.2 g thiobarbituric acid to 100 mL distilled water, which was placed in a water bath (95°C) for 60 minutes. The absorbance of the solution was then measured at 532 nm.

L. Reduced Glutathione Activity

The method described by Smith et al.,[7] with slight modifications, was used to assay reduced glutathione (GSH) activity. Samples were prepared by centrifuging 2 g liver tissue homogenate with 20 mL Tris-HCl buffer for 10,000 rpm at 4°C for 10 minutes. The absorbance of the supernatant obtained was measured at 412 nm. Then 3 mL of

0.2 M phosphate buffer (pH 8), prepared by adding 34.84 g K_2HPO_4 and 27.22 g KH_2PO_4 to 1000 mL distilled water, was added and the absorbance was measured; then 0.5 mL Ellman's reagent was added to the solution. The solution was left at room temperature for 5 minutes before the absorbance was measured again.

M. Statistical Analysis

All data and measurements obtained from the analyses were analyzed using 1-way analysis of variance to test for variance. Significance was set at $P < 0.05$ for all analyses. Minitab 17 was used to perform the analysis. A *post hoc* analysis was conducted to identify differences between 2 groups when the *P* values of 1-way analysis of variance were <0.05.

III. RESULTS AND DISCUSSION

A. Body Weight

Mean body weight of the experimental rats were measured weekly throughout the experiment. Based on the results obtained, mean body weights of the groups fed 100 mg and 250 mg of the freeze-dried *L. rhinocerotis* powder and the positive control group were increased after the fourth week of administration of the respective treatments. Unlike the other groups, the group administered 500 mg of the freeze-dried *L. rhinocerotis* powder did not experience a decrease in body weight; the mean body weight of the group increased throughout the experiment (Fig. 1), indicating that the weight increase caused by freeze-dried *L. rhinocerotis* powder is dose-dependent.

Edible mushrooms were found to contain large amounts of dietary fiber and polysaccharide-protein complexes that can help regulate weight, alleviate diabetic complications, and exert anticancer effects.[8] Because *L. rhinocerotis* is rich in dietary fiber and polysaccharides such as β-glucan, the mushroom may be useful in regulating the body weight of humans with diabetes.[2]

B. Blood Glucose Concentration

Mean blood glucose concentrations in experimental rats were measured weekly throughout the experiment. The reference values for fasting blood glucose concentrations were determined according to Wang et al.[9] The mean blood glucose concentrations of rats in the negative control group remained high (25–30 mmol/L) throughout the experiment. Those

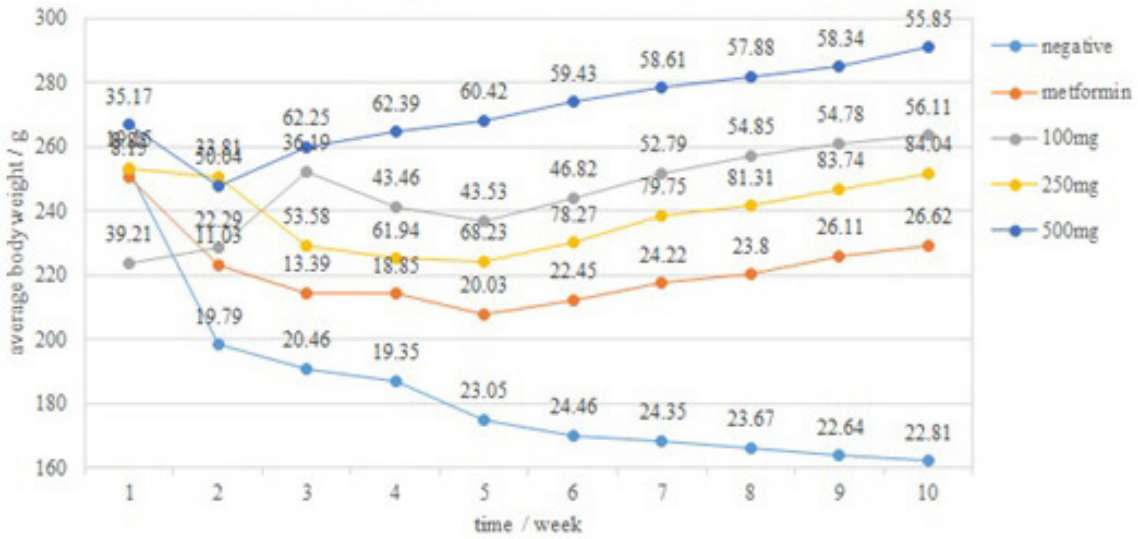

FIG. 1: Average weight (grams) of rats plotted against time (weeks). Labels at each data point show the standard deviation of each group.

of rats in the positive control group decreased from the first to the fifth week, then remained between 15 and 20 mmol/L from the sixth week until the end of the experiment. The mean blood glucose concentrations of rats in the groups fed 100 and 250 mg *L. rhinocerotis* powder also decreased throughout the experiment; however, their mean blood glucose was still >7.5 mmol/L at the end of the experiment. The mean blood glucose concentration of rats in the group fed 500 mg *L. rhinocerotis* powder decreased until the fourth week, then remained at 5–10 mmol/L until the end of the experiment. This group experienced a greater decrease in mean blood glucose concentration than that in the other groups (Fig. 2).

The mechanism responsible for regulating blood glucose concentrations may be due to β-glucan content, which is abundant in *L. rhinocerotis*. Mihailovic et al.[10] demonstrated that a β-glucan-enriched extract was able to mediate oxidative stress and lower blood glucose concentrations in STZ-induced diabetic rats. The study also showed that the β-glucan-enriched extract was able to reduce the formation of advanced glycation end products. Other supporting evidence from Hwang et al.[11] demonstrated that crude exopolysaccharides containing 2 different heteropolysaccharides and 2 proteoglycans produced from the medicinal mushroom *Phellinus baumii* exerted a hypoglycemic effect on STZ-induced Sprague-Dawley rats. Lau et al.[2] discovered that the fruiting bodies and sclerotia of *L. rhinocerotis* were rich in carbohydrates such as dietary fiber and β-glucan; they found that the β-glucan content in *L. rhinocerotis* produced a sizeable beneficial effect on diabetic complications and lowered blood glucose concentrations.

C. Other Observations

Rats in the negative control group were found to be physically inactive and thin; produced excessive urine; and excreted yellowish, semisolid, and watery feces. Rats were also frequently hunched. Wei et al.[12] described similar signs in their study of chronic STZ-induced diabetic rats; the rats were described as having high water and food intake but no increase in body weight, and there was evidence of cataract development and blindness. The rats from the metformin group were found to be more physically active after the third week, and an increase in appetite was also observed. However, the group also excreted yellowish, semisolid, and watery feces.

In our study, rats in the group fed 100, 250, and 500 mg of *L. rhinocerotis* powder were observed

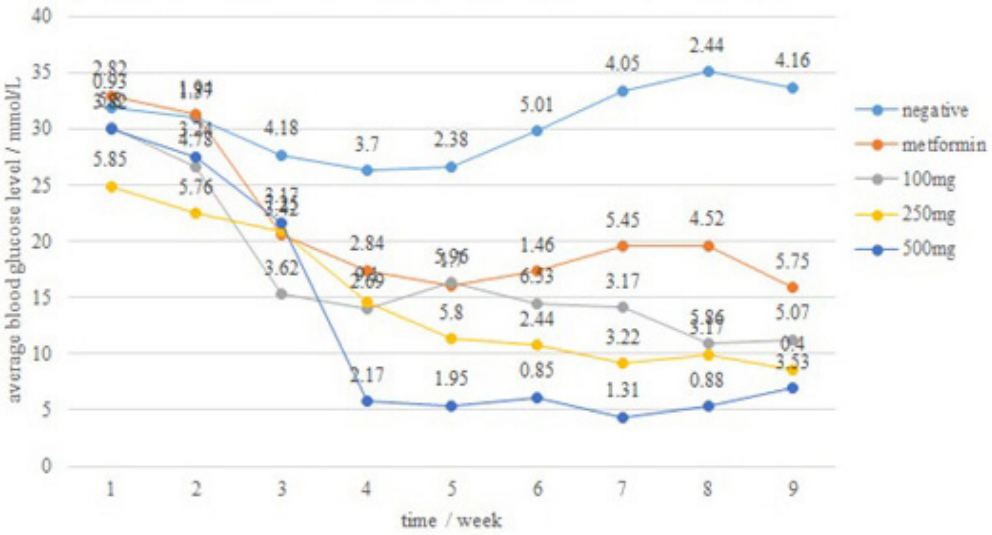

FIG. 2: Figure of average blood glucose level (millimoles per liter) of rats plotted against time (weeks). Labels at each data point show the standard deviation of each group.

to have an increased appetite and were more active after the third week. The feces produced by these rats were watery during the first and second weeks of experiment, but the condition improved after the third week. The rats excreted dark-brown, firm feces.

D. Full Blood Analysis

A rat was selected randomly from each group for the full blood analysis. The hematology reference ranges for the rats were based on the study by Petterino and Argentino-Storino[13] of normal parameters of laboratory rats. Results showed that hemoglobin in rats in the negative control group was slightly lower than that in the other groups, whereas the red blood cell counts of rats in the negative control group was similar to those of rats in the group administered 100-mg *L. rhinocerotis* powder, which was lower than the metformin and 250-mg *L. rhinocerotis* powder groups. Hence, the concentration of *L. rhinocerotis* powder administered may influence the production of hemoglobin and red blood cells. Annadurai et al.[14] discovered that glycosylated hemoglobin concentrations had a moderate to strong correlation with antioxidants, malondialdehyde, and lipid profile parameters, indicating that serum hemoglobin can be used as a diagnostic test for diabetes. The packed cell volume, mean cell volume, cell distribution width, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration of all groups were within normal ranges.

Platelet count for the negative control group was lower than that of the other treatment groups. Winocour and Colwell[15] concluded that diabetes can cause an alteration in platelet function and enhanced leukocyte-mediated fibrinolysis, which leads to a decrease in the fibrinolytic activity of the whole blood. The evidence of this occurrence can be observed in the abnormal distribution of neutrophils, lymphocytes, monocytes, and eosinophils in the negative control group (Table 1).

E. Biochemistry

The serum biochemistry parameters of the rats fed *L. rhinocerotis* powder were within normal levels. Sodium concentration were <156 mmol/L, and chloride concentrations were 90–110 mmol/L, within the normal range. Total bilirubin concentrations (<2 μmol/L for all groups) fell within the normal reference range. Serum urea concentrations in the negative control group was highest among all groups. The 100-mg *L. rhinocerotis* powder group had a serum urea concentration similar to that of the metformin group, whereas the 500-mg *L. rhinocerotis* powder group had the lowest serum urea concentration, followed by the 250-mg *L. rhinocerotis* powder group. The effect of *L. rhinocerotis* powder was similar to metformin in lowering serum urea concentration and had a more profound effect when the dose was increased.

However, *L. rhinocerotis* powder did not have an effect on creatinine metabolism; the serum creatinine concentrations of the 100-, 250-, and 500-mg *L. rhinocerotis* powder groups fall within the same range as the negative control groups, whereas the metformin group had a higher serum creatinine concentration. The serum potassium concentration was higher than the reference value in all groups of rats.

The reference values for albumin, globulin, and the albumin-to-globulin ratio were based on a study by Zaias et al.[16] Serum albumin and globulin concentrations in the negative control group were lower than those in the other groups; however, the albumin-to-globulin ratios of all groups were similar and slightly less than the reference range, which is 0.9–1.2.

The alkaline phosphatase (ALP) concentrations of the negative control and 100-mg *L. rhinocerotis* powder groups were higher than the reference values, according to the study by Petterino and Argentino-Storino.[13] The metformin group had an ALP concentration that was lower than that of the negative control group and the 100-mg *L. rhinocerotis* powder groups, but slightly higher than that of the 250- and 500-mg *L. rhinocerotis* powder groups. This indicates that *L. rhinocerotis* powder is able to cause a decrease in ALP concentrations at a higher dose, and it seemed to be more effective than metformin. An elevated ALPconcentration is usually associated with liver or bone disorders.[17]

TABLE 1: Hematology of Sprague-Dawley Rats in Different Treatment Groups

Parameters	Reference Range	Groups					
		Control	Negative Control	Metformin	*L. rhinocerotis* Powder		
					100 mg	250 mg	500 mg
Hemoglobin (g/L)	161 ± 7.0	161	156	176*	161	160	170*
RBC (10^{12}/L)	8.4 ± 0.4	8.40	8.82	9.12*	8.40	9.45	9.84
PCV (L/L)	—	0.48	0.47	0.56*	0.50	0.49	0.52
MCV (fl)	56.7 ± 1.8	57	53*	61*	59*	52*	52*
MCH (pg)	19.0 ± 0.5	19	18*	19	19	17*	17*
MCHC (g/L)	336.0 ± 11.0	336	334	317*	325	326	332
RDW (%)	—	12.9	17.0*	15.8*	16.1*	15.3*	16.6*
WC count (10^9/L)	12.9 ± 2.4	13	7.0*	3.7*	6.3*	4.7*	5.1*
Neutrophils (%)	11.3 ± 5.3	11	18*	34*	29*	23*	21*
Lymphocytes (%)	83.1 ± 5.4	83	79	58*	61*	70*	67*
Monocytes (%)	2.5 ± 0.8	3	1*	3	5*	4*	4*
Eosinophils (%)	1.3 ± 0.7	1	2	5*	5*	3*	8*
Platelets (10^9/L)	1028.8 ± 153.0	1028	588*	796*	672*	779*	793*

MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean cell volume; PCV, packed cell volume; RBC, red blood cell; RDW, cell distribution width; WC, white cell.
*Significant difference compared with the control.

The serum γ-glutamyl transferase concentration of the negative control group was higher than that of the other groups. *L. rhinocerotis* powder was found to have the same effect as metformin on decreasing serum γ-glutamyl transferase concentrations, as the results obtained from all the groups were the same. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations of the 100-, 250-, and 500-mg *L. rhinocerotis* powder groups were significantly lower than those of the metformin group and the negative control (Table 2).

F. Lipid Studies

Based on the results obtained, the total cholesterol–to–high-density lipoprotein ratios of all groups were similar. Also, metformin did not have a significant effect in reducing serum cholesterol concentrations. The readings from the metformin group corresponded to the findings of Wulffelé et al.,[18] who stated that metformin did not have an intrinsic effect on high-density lipoprotein cholesterol and triglyceride levels in people with diabetes, and that it has a minimal effect on total and low-density lipoprotein cholesterol levels.

The triglyceride levels of the 250- and 500-mg *L. rhinocerotis* powder groups were lower than that of the negative control. *L. rhinocerotis* powder had a slight effect in modulating the serum triglycerides, similar to that of metformin, and the effect was dose-dependent. Jeong et al.[19] reported a similar trend in their study of the effects of the white button mushroom (*Agaricus bisporus*) on diabetic rats. The white button mushroom did not affect the plasma cholesterol concentrations of the diabetic rats, but it did reduce plasma triglyceride, ALT, and AST concentrations (Table 3).

TABLE 2: Biochemistry of Sprague-Dawley Rats in Different Treatment Groups

Parameter	Reference Value	Group					
		Control	Negative Control	Metformin	*L. rhinocerotis* Powder		
					100 mg	250 mg	500 mg
Sodium (mmol/L)	146.6 ± 9.0	139	132	140	131	145	141
Potassium (mmol/L)	3.9 ± 0.5	6.1	7.3*	8.5*	7.5*	6.7	7.2*
Chloride (mmol/L)	96.3 ± 3.9	101	93*	99	91*	104	101
Urea (mmol/L)	14.7 ± 3.7	5.6	8.8*	7.8*	7.7*	6.8	6.2
Creatinine (mmol/L)	44.2 ± 8.8	57.8	46*	58	54	42*	50
Uric acid (mmol/L)	—	0.14	0.35*	0.36*	0.21	0.28*	0.35*
Total protein (g/L)	68.0 ± 4.0	75.4	58*	75	64*	74	78
Albumin (g/L)	35.0 ± 4.0	38.2	28*	36	30*	36	33
Globulin (g/L)	35.0 ± 4.0	37.2	30*	39	34	38	45*
A:G ratio	1.0 ± 0.1	1.0	0.9	0.9	0.9	0.9	0.7*
ALP (U/L)	340.6 ± 113.5	156.2	835*	425*	712*	272	388*
Total bilirubin (μmol/L)	1.71 ± 0.1	<2	<2	<2	<2	<2	<2
GGT (U/L)	1.2 ± 0.9	<3	5*	<3	<3	<3	<3
AST (U/L)	92.6 ± 31.3	129.8	502*	321*	174*	141	297*
ALT (U/L)	44.8 ± 11.4	55.4	296*	161*	78*	77	116*

A:G ratio, albumin-to-globulin ratio; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; GGT, γ-glutamyl transferase.
*Significant difference compared with the control.

TABLE 3: Serum Lipid Profile of Sprague-Dawley Rats in Different Treatment Group

Parameters	Reference Range	Group					
		Control	Negative Control	Metformin	*L. rhinocerotis* Powder		
					100 mg	250 mg	500 mg
TC (mmol/L)	<5.2	1.4	1.1	1.5	1.3	1.8*	1.2
TG (mmol/L)	<1.68	0.57	0.94*	0.60	0.87*	0.67	0.62
HDL (mmol/L)	>1.03	1.08	0.35*	0.51*	0.45*	0.58*	0.36*
LDL (mmol/L)	<2.58	0.12	0.32*	0.71*	0.45*	0.91*	0.56*
TC:HDL ratio	<5.0	1.3	3.1*	2.9*	2.9*	3.1*	3.3*

TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
*Significant difference compared with the control.

G. Analyses of Oxidative Stress Biomarkers

1. GSH Assay

We observed a general increase in GSH concentrations in rats administered 500 mg *L. rhinocerotis* powder; the GSH activity in these rats seemed to be greater than that of the negative control as the dose was increased. Statistical analysis showed that only the 500 mg/kg concentration of *L. rhinocerotis* had a significant effect on GSH activity; metformin and 100 and 250 mg *L. rhinocerotis* powder did not obviously increase the GSH activity in tissues (Table 4).

The increase in GSH activity in the rats fed *L. rhinocerotis* powder may be attributed to the mushroom's nutrient composition. According to Yap et al.,[20] *L. rhinocerotis* contains amino acids that are essential for GSH formation, such as cysteine, glycine, methionine, and glutamate. It is possible that supplementation of these amino acids stimulated GSH formation and activity.

2. SOD Assay

No significant difference was found between the effects of metformin and the 100 mg/kg and 250 mg/kg concentrations of *L. rhinocerotis* powder. Only 500 mg *L. rhinocerotis* powder produced a significant effect on SOD activity (Table 4). According to a study by Lau et al.,[2] minerals essential for SOD formation (e.g., manganese, iron, and zinc) were abundant in *L. rhinocerotis*, and this may be the key to the increased SOD activity in the liver tissue of rats.

3. CAT Assay

Administration of *L. rhinocerotis* powder significantly increased CAT activity, and the effect was dose-dependent. Based on the statistical analysis conducted in this study, administration of 100 mg *L. rhinocerotis* powder did not produce a significant difference in CAT activity. However, the effect of metformin on CAT activity was similar to that in the groups fed with 250 and 500 mg *L. rhinocerotis* powder (Table 4).

H. Lipid Peroxidation

Based on the results obtained, both metformin and *L. rhinocerotis* powder were able to reduce LPO. However, the effect of 100 and 250 mg/kg *L. rhinocerotis* did not statistically significantly reduce LPO, whereas metformin and 500 mg/kg *L. rhinocerotis* powder significantly reduced LPO. The results indicate that the effect of *L. rhinocerotis* powder on LPO was dose-dependent (Table 4).

TABLE 4: Oxidative Stress Biomarkers' Assays of Liver Homogenates from Streptozotocin-Induced Diabetic Sprague-Dawley Rats

Group	Rats/ Group (n)	GSH Activity (nmol/mg)	SOD Activity (nmol/mg)	CAT Activity (mmol/mg)	Lipid Peroxidation (nmol/mg)
Negative control	5	1.088 ± 0.486^{a}	0.1054 ± 0.0295^{a}	0.0465 ± 0.0777^{a}	0.2562 ± 0.1346^{a}
Metformin	5	1.309 ± 0.492^{ab}	0.1423 ± 0.0376^{ab}	0.0876 ± 0.0148^{b}	0.1220 ± 0.0221^{b}
L. rhinocerotis powder					
100 mg	5	1.319 ± 0.276^{ab}	0.1439 ± 0.0317^{ab}	0.0732 ± 0.0209^{ab}	0.1860 ± 0.0821^{ab}
250 mg	5	1.500 ± 0.720^{ab}	0.1306 ± 0.0224^{ab}	0.0917 ± 0.0255^{b}	0.1614 ± 0.0308^{ab}
500 mg	5	1.947 ± 0.417^{b}	0.1573 ± 0.0381^{b}	0.0829 ± 0.0205^{b}	0.1098 ± 0.0296^{b}

Data are mean ± standard deviation unless otherwise indicated. Different superscript letters indicate a significant difference between the groups at $P < 0.05$, as measured by the Fisher least significant difference method.

IV. CONCLUSIONS

The results presented here show that freeze-dried *L. rhinocerotis* powder possesses antidiabetic properties comparable to those of the antidiabetic drug metformin. Analyses of oxidative stress biomarkers also show that there were significant increases in GSH and CAT activities, a slight increase in SOD activity, and a decrease in LPO. The effect of *L. rhinocerotis* powder was found to be dose-dependent and to have a regulatory effect on the hematology of diabetic rats. Supplementation of *L. rhinocerotis* powder regulated whole-blood components to a near normal state compared with the negative control. *L. rhinocerotis* was able to normalize serum potassium, urea, and creatinine concentrations. The albumin and globulin concentrations of the rats administered the mushroom powder was normalized. Indicator enzymes such as ALP, AST, and ALT were regulated. This study showed that *L. rhinocerotis* may be used as an alternative to treat diabetes and its complications. More research could be done regarding the beneficial effects of this mushroom.

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