

Antihyperglycemic Activity of Edible Mushroom, *Lentinus edodes* in Alloxan Induced Diabetic Swiss Albino Mice

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ABSTARCT

Consequences a cure for diseases and infections using herbal medicine are primordial approach. The present study aimed at investigation of anti-hyperglycemic activity of 50% ethanolic extract of edible mushroom, *Lentinus edodes*, in alloxan induced diabetic Swiss Albino Mice (SAM). There are a few unequivocal studies to authorize in-vitro anti-diabetic activity of edible mushroom. This prospective study was designed to comprehend the hypoglycemic properties of edible mushroom on the diabetes mellitus. Seven groups of SAM consisting of each group with five male SAM were used in this experiment and diabetes in each SAM was induced by intraperitoneal injection of 180 mg/kg alloxan excepting group-VII (G_VII). The group-I (G_I) served as a negative control and received a daily intraperitoneal injection of normal saline solution (1 ml / 100g of 0.9 % NaCl, V/W), and group-II (G_II) also assisted as positive control receiving a daily intraperitoneal injection of metformin (3.33 mg/Kg), and the subsequent groups of SAM namely group-III(G_III), group-IV(G_IV), group-V(G_V), and group-VI (G_VI) were received mushroom extract/daily by using intragastric feeding tube. The ethanolic extract of *Lentinus edodes* at the dose of 200, 400, 600 and 800 mg /kg body weight was administered orally once a day to the groups of G_II, G_IV, G_V, and G_VI for 3 days and then the fasting blood glucose was estimated in both normal and alloxan induced diabetic SAM. The fasting blood glucose was found to be significantly reduced ($p < 0.05$) in treated SAM in compared to control SAM groups. The study discloses that *Lentinus edodes* has significant antidiabetic activity in alloxan induced diabetic SAM. So, The *Lentinus edodes* extract appears promising for the development of a phytomedicine for diabetes mellitus.

Key Words: Hypoglycemia, *Lentinus edodes*, edible mushroom, phytomedicine

INTRODUCTION

Diabetes mellitus (D.M) is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Various complications develop because of the metabolic derangements in diabetes, many of these results to diseases of blood vessels either large (macrovascular disease) or small (microangiopathy).¹ Dysfunction of vascular endothelium is an early and critical event in the development of vascular complications.² Macrovascular disease consists of accelerated atheroma which is much more common and severe in diabetic patients.³ Microangiopathy is a distinctive feature of diabetes mellitus and particularly affects the retina, kidney and peripheral nerves^{4,5}; thus, treatments of diabetes mellitus is necessary to achieve as near normal blood glucose levels as possible either through Diet by increased consumption of soluble fibers, unrefined carbohydrate, or through administration of oral hypoglycemic drugs, or Insulin. Currently an attempt was made to collect traditional medicine concerning the treatments of diabetes, as part of this effort, multitudes of plants were being studied worldwide to check their possible hypoglycemic effect^{6,7} such as turmeric root, fenugreek seeds, bitter melon, green tea, bay leaves⁸.

Mushrooms are also exemplary sources of natural medicines with anti-diabetic activity⁴. Extracts of edible mushrooms have been proved to be the ideal food for diabetic prevention of hyperglycemia owing to their high content of fibre and protein and low fat content⁵. Various mushrooms have been reported to possess hypoglycemic activity^{5,8}. The compounds seem to mop the free radicals generated in the normal natural metabolism of aerobic cells, mostly in the form of reactive oxygen species (ROS). These include superoxide (O₂⁻) and hydroxyl (OH⁻) radicals among several others. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides⁹. Once in circulation, most of the free radicals are neutralized by cellular antioxidant defense enzymes e.g. Superoxide dismutase (SOD) or catalase (CAT)^{10,12}. Non-enzymatic molecules like ascorbic acid and carotenoids are reported to be present in mushrooms and they also act as antioxidants^{13,14}. Maintenance of equilibrium between free radicals production and antioxidant defenses is an essential condition for normal organism functioning^{15,16}. In this study attempts were made to investigate the anti-diabetic effect of the aqueous extract of *Lentinus edodes* in alloxan induced diabetic Swiss Albino Mice (SAM).

Table 1: Effects of ethanolic extract of *Lentinus edodes* on body weight of alloxan induced diabetic mice

Groups	Dose mg/Kg	Body weight of SAM (gram)				Two-tailed p value
		0 hrs Mean±SEM	24 hrs Mean±SEM	48 hrs Mean±SEM	72hrs Mean±SEM	
G_I	0.2ml normal saline	32.00±0.03	30.80±0.02	29.40±0.04	27.40±0.03	0.005
G_II	3.33 mg metformin	33.20±0.02	33.60±0.03	34.00±0.03	35.20±0.04	0.002 #
G_III	200 mg sample extract	33.00±0.06	33.80±0.05	34.40±0.05	34.80±0.02	0.005
G_IV	400 mg sample extract	32.20±0.05	4.20±0.04	35.00±0.03	35.40±0.01	0.002 #
G_V	600 mg sample extract	32.40±0.05	34.80±0.03	35.60±0.03	36.20±0.02	0.005
G_VI	800 mg sample extract	32.20±0.04	35.20±0.01	35.80±0.02	36.20±0.06	0.002 #
G_VII	General group	34.00±0.03	35.20±0.07	35.80±0.01	36.60± 0.03	0.005

Legends: SAM= Swiss Albino Mice, mg=milligram, SEM= Standard Error of Mean, *p 0.05 calculated from Mann Whitney test, and significant from G_I, = Gaussian approximation p value, # = exact p value

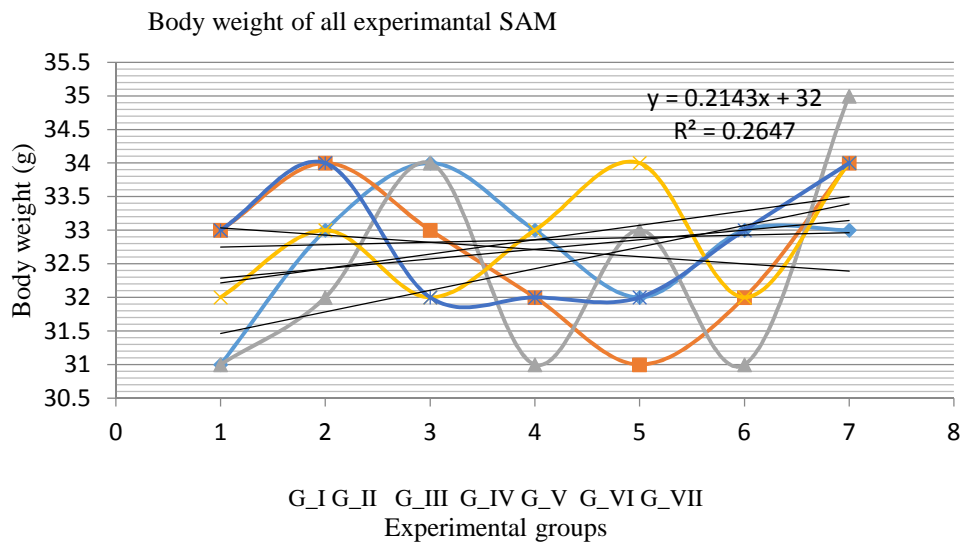


Figure 1: Body weight of all diabetic SAM (G_I to G_VI) and non-diabetic SAM (G_VII) at the beginning of experiment.

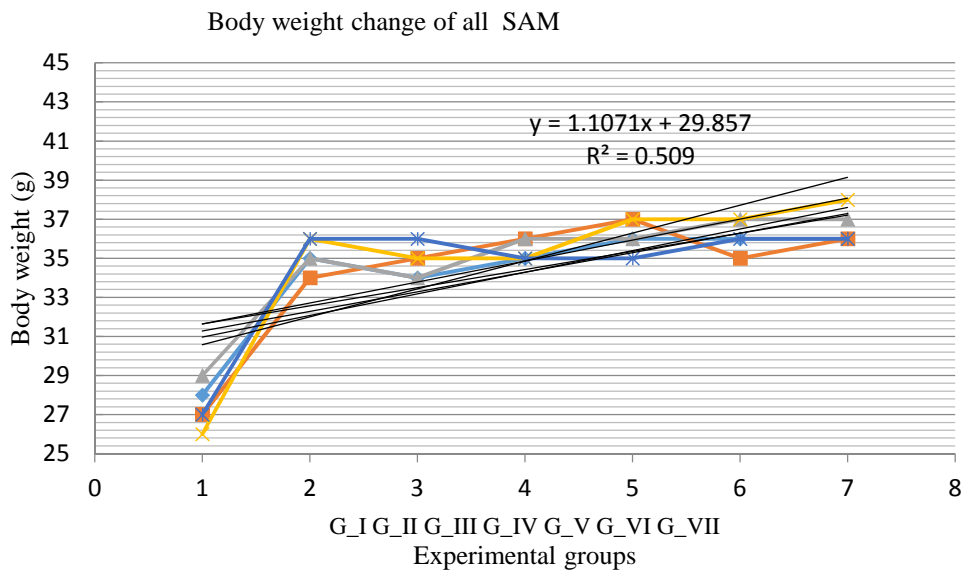


Figure 2: Body weight change of all diabetic SAM (G_I to G_VI) and non-diabetic SAM (G_VII) at the end of experiment

MATERIALS AND METHODS

Sample collection and preparation: At first with the help of a comprehensive literature review Shiitake mushrooms was selected for investigation and then 600 gm of the fresh Shiitake mushrooms (*Lentinus edodes*) was collected from National Mushroom Development & Extension Centre, Sobhanbag, Savar, Dhaka-1340, Bangladesh. It was identified by specialized authority. The whole mushrooms was cut perpendicularly into small pieces and dried by shed drying process having a good air circulating system for fifteen days. The dried mushrooms were ground up to coarse powder with a mechanical grinder (Grinding Mill). Then the powdered sample was kept in clean air tight container till extraction. The weights of the total dry powder were 23 gm.

Extraction: About 20 gm of mesh powder of *Lentinus edodes* was taken in a clean flat bottom flask and soaked in 200ml of 50% ethanol. The flask was sealed by Aluminum foil and kept for a period of 2 days accompanying occasional shaking and stirring. The whole mixture was then filtrated through cotton in funnel. The filtrate extract thus obtained and kept in room temperature in a beaker with protective measured from dust and the solvent was allowed to evaporate by rotary evaporator and crude extract was collected for further uses.

Drugs and Chemicals: Alloxan monohydrate was purchased from BDH Chemicals, Poole, England. The chemicals used were of analytical grade.

Experimental mice: Thirty five (35) healthy Swiss albino mice, SAM, (males, weight 31-34 g) were collected from the Animal Resources Branch of The International Center for Diarrhoeal Research, Dhaka, Bangladesh. All animals were divided into seven groups (six experimental groups and one general group). They were housed in plastic cages having dimension of (28×22×13 cm). Soft wood shavings were used as bedding of cages. Animals were maintained under standard environmental conditions (temperature: 24.0±1.0C), relative humidity: 55-65% and 12 hrs light/12 hrs dark cycle). Husk and excreta were removed from the cages as per 2-3 days. Pellets of mice foods, provided by ICDDRB were given to the mice with fresh tap water. The newly bought mice were given 10 days rest to get over the food and water restrictions incurred during transit and to get themselves adapted with the new environment of the laboratory, before being employed in the experiment. Animal studies were attributed accordingly to the Institute Animal Ethics Committee Regulations authenticated by the committee for the purpose of control and supervision of experiment on animals of Primeasia University, Bangladesh.

Experimental Induction of Diabetes: The thirty (30) mice were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 180 mg/kg body weight. Blood samples were collected before the administration of alloxan and after 7 days of alloxan administration. Diabetic state was confirmed when the blood sugar level was above 200 mg/dl. The mice with moderate diabetes were used for the experiment.

Animal Allotment: After induction of diabetes, the thirty (30) mice were divided in to a six different groups. The

G_I to G_VI were diabetic groups, and G_VII was general group.

- Group-I (G_I): Negative control mice received normal saline and fed on normal diet.
- Group-II (G_II): Positive control mice received metformin (3.33 mg/Kg).
- Group-III (G_III): Mice were treated with 200 gm/Kg of body weight daily by using intragastric tube for 3 days.
- Group-IV (G_IV): Mice were treated with 400 gm/Kg of body weight daily by using intragastric tube for 3 days.
- Group-V (G_V): Mice were treated with 600 gm/Kg of body weight daily by using intragastric tube for 3 days.
- Group-VI (G_VI): Mice were treated with 800 gm/Kg of body weight daily by using intragastric tube for 3 days.
- Group-VII (G_VII): General group of mice were fed daily on normal diet.

The body weight change and fasting blood glucose level of all SAM in each group were recorded at regular intervals during experimental period. At the end of 24 hrs, 48hrs, and 72 hrs blood was collected by tail tipping method and the mice blood was plummeted on the dextrostix reagent pad. This pad was introduced into microprocessor digital blood glucometer and the readings were documented⁹.

Statistical analysis: Expressive statistical analysis of the data were completed using GrapPad Prism⁵. Differences were measured significant if $p < 0.05$, calculated from Mann Whitney test.

RESULTS

Table 1 showed that there were observable body weight change of extract treated diabetic SAM in compared to negative diabetic control SAM (G_I) and general SAM group (G_VII). Treatment of diabetic SAM with the extract of edible mushroom, *Lentius edodes* upgraded weight gain compared to untreated diabetic SAM. Body weight change in each SAM was noticeable in all experimental groups. Results were shown in figure 1 and in figure 2.

In figure 1, the body weight of each diabetic SAM in G_I, G_II, G_III, G_IV, G_V, G_VI and each non-diabetic SAM in G_VII was documented at the beginning of experiment and the lowest documented body weight of SAM was 31 g following highest of 35 g. The mean body weight of all experimental groups was also noted and shown in table 1. This figure 1 also describe the range of SAM body weight and body weight of all diabetic SAM was 31, 33, 31, 32, and 33 g in G_I SAM, 33, 34, 32, 33, and 34 g in G_II, 34, 33, 34, 32, and 32 g in G_III, 33, 32, 31, 33, and 32 g in G_IV, 32, 31, 33, 34, and 32 g in G_V, 33, 32, 31, 32, and 33 g in G_VI and body wt of SAM in general group was 33, 34, 35, 34 and 34 g.

The body weight of treated diabetic SAM, non-treated diabetic SAM, G_I, and normal SAM, G_VII were recorded and verified at the end of experiment, 3rd day, and the mean body weight on 3rd day was shown in table 1 but figure 2 charted with the body weight of each SAM during this experiment. It was cleared that the mean body weight of untreated diabetic, G_I, SAM was decreased to 27.40 g (-14.37 %) but body weight of each SAM in treated diabetic groups was increased as dose dependent manner.

Table 2: Changes in fasting blood glucose levels of general and experimental SAM

Groups	Dose mg/Kg	Fasting blood glucose level (mmol/L)				Two-tailed p value
		Before treatment Mean±SEM	24 hrs after treatment Mean±SEM	48 hrs after treatment Mean±SEM	72 hrs after treatment Mean±SEM	
G_I	0.2ml normal saline	31.40±0.03	31.80±0.05	31.80±0.02	31.54±0.03	0.005
G_II	3.33 mg metformin	31.60±0.04	15.80±0.03	12.60±0.04	9.14±0.02	0.002
G_III	200 mg sample extract	31.08±0.06	29.20±0.04	25.40±0.03	24.20±0.05	0.005
G_IV	400 mg sample extract	30.00±0.02	23.80±0.03	22.20±0.05	21.60±0.03	0.002
G_V	600 mg sample extract	30.00±0.03	22.40±0.01	20.00±0.03	17.20±0.02	0.005
G_VI	800 mg sample extract	31.40±0.04	21.40±0.04	18.20±0.05	15.20±0.03	0.002
G_VII	General group	8.28±0.03	8.20±0.02	8.15±0.04	8.25±0.06	0.005

Legends: SAM= Swiss Albino Mice, mg=milligram, SEM= Standard Error of Mean, *p 0.05 calculated from Mann Whitney test, and significant from G_I, = Gaussian approximation p value

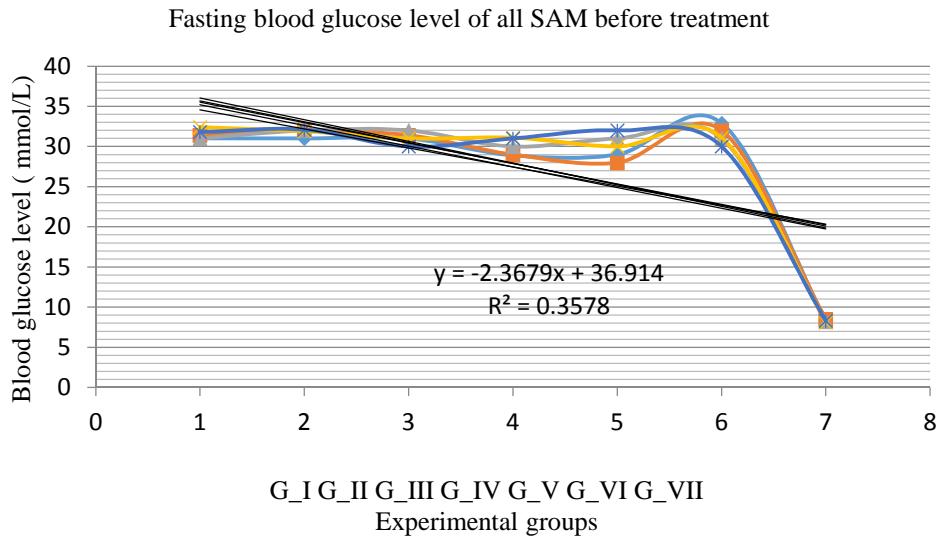


Figure 3: Fasting blood glucose level of each SAM in all experimental groups before treatment.

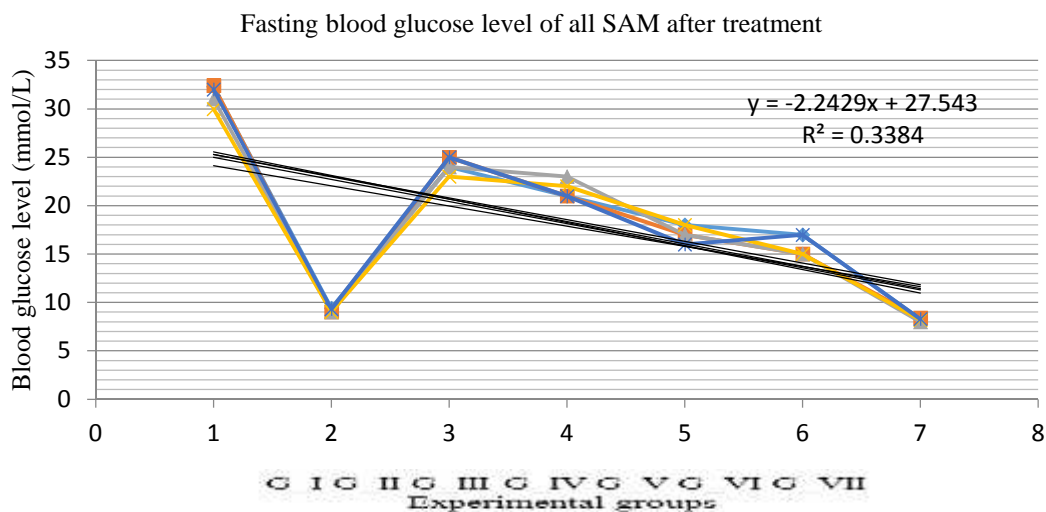


Figure 4: Fasting blood glucose level of each SAM in all experimental groups after treatment on 3rd day of experiment.

The noted mean body weight of dose 200 mg (G_III), 400 mg (G_IV), 600 mg (G_V), and 800 mg (G_VI) treated experimental group was 34.80 g (5.45%), 35.40 g (9.93%), 36.20 g (11.72%), and 36.20 g (12.42%) respectively on 3rd day of experiment. The mean body weight change of each group at 24 hrs, and 48 hrs experimental intervals was also documented and enlisted in table 1.

The effects of *Lentinus edodes* extract on glucose tolerance in diabetic SAM are shown in table 2. A dose-dependent reduction of blood glucose level in alloxan induced SAM was recorded and observed significant ($p < 0.005$) reduction in blood glucose level of diabetic SAM with period of acute study compared to control SAM. Three days of daily treatment of four different dose of extract namely 200 mg (G_III), 400 mg (G_IV), 600 mg (G_V), 800 mg (G_VI) in each SAM lead to a dose-dependent reduction of blood glucose level by 24.20 mmol/L (22.13%), 21.60 mmol/L (28.0%), 17.20 mmol/L (42.66%), and 15.20 mmol/L (51.59%). There were no change blood glucose level in negative control SAM, G_I, but found to be significantly decreased body weight during 3rd day of experiment.

The hypoglycemic activity of *Lentinus edodes* extract on fasting blood glucose level of all alloxan induced diabetic SAM is shown in figure 3, and figure 4. Figure 3 explain the blood glucose level of all diabetic, G_I, G_II, G_III, G_IV, G_V, G_VI, SAM and non-diabetic, G_VII, SAM before treatment but in figure 4 results are presented after 72 hrs of treatment. In general group, G_VII, blood glucose level of all SAM was remained fairly constant level even if there were increased their body weight significantly but in negative control group, G_I, SAMs were lost their body weight with unchanged diabetic level. In the same time, extract treated diabetic SAMs were gained up weight with reducing blood glucose level significantly ($p < 0.05$). Blood glucose level in metformin treated SAM was most significantly decreased, G_II, documenting in table 2 and figure 4 explained it more clearly and decreased body weight was restored to general SAM group.

DISCUSSION

The management of diabetes with the artificial agent like metformin has unavoidable health effects. To avoid this health effect of diabetes treated pharmaceutical drugs, increase attention and demand of natural products with antihyperglycemic activity. In the nimble of results, our study indicates that ethanolic extracts of *Lentinus edodes* has good hypoglycemic activity. Valuation of antihyperglycemic activity of *Lentinus edodes* extract was conceded out in alloxan induced diabetic Swiss Albino Mice. The tenacity of choosing alloxan monohydrate was known to produce diabetes mellitus irreversibly by single dose of administration. The necrotic action of alloxan on the beta cell of pancreas resulting reduced production of insulin leads to metabolic aberrations in SAM like increased blood glucose level.

In diabetic patients, blood glucose does not enter into cell due to loss of insulin activity because of insulin helps to bind of glucose at extracellular part of glucose transporter

in the target cell in body. As for level of blood glucose is increased over the normal value and for this circumstances patient faces various metabolic aberrations. Because of glucose is not only energy supplier but also acts as provider of raw material for nucleic acid synthesis. Without nucleic acid synthesis cells are not to be grown resulting patient become underweight condition. The alloxan induced diabetic mice of G_I became underweight during intervals of experiment. The mean body weight of G_I SAM was 32 g at the beginning of experiment, 0th hr, but at the end of experiment weight became 27.40 g because of glucose level of all SAM in G_I remained hyperglycemic condition, 31.4 mmol/L. So, the beta cell of mice pancreases has destroyed with the acute glucose toxicity in G_I SAM resulting insulin secretion become absent. On the other hand, diabetic SAM in group G_III, G_IV, G_V, G_VI treated with the ethanolic extract of *Lentinus edodes*, and then the hypoglycemic effect of *Lentinus edodes* was observed in the three intervals of experiment like 24 hrs, 48 hrs, and 72 hrs. This observation was accomplished by collecting mice blood of above assigned intervals and then the estimated blood glucose level reveals that mushroom extract acts as hypoglycemic agent. We have documented 31.08 mmol/L, 29.20 mmol/L, 25.40 mmol/L, and 24.20 mmol/L of blood glucose in G_III SAM at 0th hr, 24 hrs, 48 hrs, and 72 hrs of experiment. This group SAM was treated with 200 mg of *Lentinus edodes* extract and lowered the blood glucose level of 22.13% and body weight was restored to normal mice. Results are shown in table 1 and 2. The SAM in G_VI were treated with 800 mg of *Lentinus edodes* extract and estimated blood glucose level was 15.20 mmol/L at 72 hrs and body weight re-established, 36.20 g, to almost normal mice body weight, 36.40 g. So, the hypoglycemic effect of *Lentinus edodes* extract is dose and time of treatment dependent and it is found that ethanolic extract of mushroom at high dose (800 mg/Kg) is more effective than at low dose (200 mg/Kg) after 72 hrs of treatment. Hence the above discussion reveals that *Lentinus edodes* extract at high dose is more effective and shows similar curative effect as standard that was metformin at 3.33 mg/Kg. This could be because of some beta cells are still surviving to act upon by *Lentinus edodes* extract to employ its insulin realising activity. In this study, sub-acute treatment with *Lentinus edodes* extract for 72 hrs instigated significant lessened in blood glucose of treated diabetic SAM compared to untreated diabetic SAM and this was also followed by a significant increase in body weight of the treated SAM. In diabetic patient, high blood glucose level has played negative activity to maintain blood homeostasis. This aberrated blood homeostasis is characterized with high osmotic pressure resulting increased blood pressure. This condition was alleviated by the treatment of the diabetic SAM with ethanolic extract of edible mushroom, *Lentinus edodes*. From the overhead discussion it decided that ethanolic extract of *Lentinus edodes* at high dose, 800 mg/Kg displayed more significant anti-diabetic activity than extract at low dose like 200 mg/Kg, 400 mg/kg, 600 mg/kg in alloxan induced diabetic SAM. This ratification vindicates its use in ethnomedical remedy for the management of diabetes mellitus.

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Conflict of Interest: We declare that we have no any conflict of interest.

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