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Assessment of anti-diabetic potential of *Lentinula edodes* using diabetes screening tools, including glycemic index and glycemic load

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ABSTRACT

Changes in dietary practices like overconsumption of energy-dense foods and a lower intake of fruits and vegetables have been recognized as primary contributors to the globally rising incidence of lifestyle-related diseases such as diabetes and cardiovascular disease. The usage of plant-based foods has been shown to reduce the risk of such chronic diseases. In this direction, the current study's objective was to conduct an *in-vivo* evaluation of the anti-diabetic potential of five different strains of the medicinal mushroom *Lentinula edodes* in healthy adult Sprague Dawley rats by using screening tools such as the glycemic index (GI) and Glycemic load (GL). For this, 36 rats were distributed into six groups, fasted overnight, and then given oral administration of glucose as a reference to one group, and each of the five different strains of the test mushroom to the rest of the five groups. Blood samples were drawn from the tail vein before (zero minutes) and after administration of either glucose or the different strains of the mushroom at 15, 30, 45, 60, 90, and 120-minute intervals. The results showed that the lowest GI values were found at 13.2% in strain DMRO-356, followed by 13.3% in strain DMRO-388, 14.8% in DMRO-34. 15.9% in DMRO-35, and 17.2% in DMRO-623; all values falling under the low GI category. Furthermore, the glycemic load (GL) of the mushroom strains revealed that DMRO-356 had the lowest GL value (7.53%), followed by DMRO-388 (8.82%), DMRO-35 (8.92%), DMRO-623 (10.24%), and DMRO-34 (10.25%). Among the five tested mushroom strains, both the GI value (13.2%) and the GL value (7.53%) were found to be the lowest in the strain DMRO-356. Since lower GI and GL values have a better response for glucose absorption in the blood sample, the present study results indicate that these different strains of *L. edodes* may be beneficial for diabetic patients and thus may also help prevent human diseases such as cardiac problems.

Keywords: Diabetes; Glycemic Index; Glycemic Load; *in-vivo* study; *Lentinula edodes*

Health is crucial to one's quality of life, and eating habits largely determine it. Chronic disorders like cardiovascular disease, cancer, obesity, and Diabetes mellitus can be prevented by eating the right foods. Diabetes mellitus is a worldwide health concern that affects people of all ages (Rai and Sohi, 1998). Diabetes is a disease marked by elevated blood sugar levels that are often managed by adopting healthy eating habits, primarily low glycemic index foods (Kharroubi *et al.,* 2015). Diabetes is a common disease in India that causes the â cells of islets of Langerhans to degenerate and become inactive. This disorder significantly affects glucose, lipid, and protein metabolism (Bahl, 2000). Diabetes can be classified into two categories. Insulin-dependent diabetes mellitus (IDDM) is a kind of diabetes that is most commonly diagnosed in children. The body produces little or no insulin in this disease, requiring daily insulin injections.

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Type 2 diabetes, also known as noninsulin-dependent diabetes mellitus (NIDDM), is the most prevalent type of diabetes, accounting for more than 90% of all diabetes cases and usually develops in adults. Diet, exercise, or oral anti-diabetic medicines may be enough to treat high blood sugar levels in this kind of diabetes (Visanthamein and Savita, 2001).

Food and lifestyle are critical elements in affecting or promoting health throughout one's life. Food price cuts and their easy access, as well as sedentary lifestyles and cooking time constraints, are responsible for "nutritional transition" (Vorster *et al.*, 1999; Popkin 2004, 2006, 2009; Astrup *et al.*, 2008), which is characterized by a gradual shift from decreased consumption of plant-based foods (primitive food) such as fruits, vegetables, and whole grains to increased intake of high-fat and energy-dense, packaged junk, animal-based foods (WHO, 2000; Popkin, 2001; Popkin 2006, 2009; Astrup *et al.*, 2008). Furthermore, it is related negatively to health and has been strongly linked to the increasing global incidence of diseases such as obesity, diabetes, and heart disease, all of which are linked to the consumption of high glycemic index foods and eating habits (Ludwig 2000; WHO/FAO, 2002; Popkin 2004, 2006, 2009; Brand-Miller, 2004; Jenkins *et al.*, 2006; Ma *et al.*, 2006; Astrup *et al.*, 2008).

The glycemic index (GI) is a nutritional monitoring system that measures how quickly it elevates blood sugar following two or more hours after food intake (postprandial glycemia). It has medically important advantages in preventing and managing diabetes, heart disease, various cancers, and obesity (Jenkins *et al.*, 2002; Brand-Miller *et al.*, 2003; Anon, 2006). Food containing carbohydrates are classified as high, intermediate, or low GI, based on how quickly the rise in blood sugar levels (Mendosa, 2000) is associated with the rate of digestion and absorption of sugars and starches in that food (FAO/UN, 1998). A high GI food has a GI of more than 70%, and will quickly digest food during digestion and absorption, causing a rapid rise in blood sugar levels but only for a short time, whereas low GI foods, with a GI of less than 55%, will slowly digest and release glucose into the bloodstream, as will foods with an intermediate GI range of 56% to 69%. Low GI foods respond better to postprandial glycemia, leading to a slow and gradual rise in circulating insulin and gastrointestinal hormone levels; as a result, feeling of fullness increases, resulting in a decrease in food consumption; this may benefit patients with diabetes, obesity, and cardiovascular disease (CVD) (Jenkins *et al.*, 2002; Bornet *et al.*, 2007). On the other hand, high GI foods are responsible for increasing insulin secretion, which causes postprandial hyperinsulinemia and an increase in the feeling of hunger and voluntary food consumption.

The Glycemic Load (GL) is calculated using the total amount of carbohydrates in a particular quantity of food. The GL system (Vega-López *et al.,* 2018) similarly divides food into three categories: High GL: 20 and higher indicates that a food has a large impact on blood sugar levels, whereas low GL: 10 and less suggests that a food has just a slight effect on blood sugar levels, and medium GL: 11–19 indicates that a food is having a minor impact on blood sugar levels. The glycemic index and glycemic load have risen in recent nutrition transition, primarily a carb-rich diet responsible for it (Ludwig, 2002). The dietary glycemic index measures carbohydrate quality, representing the impact on blood glucose, while the dietary glycemic load includes carbohydrate quality and quantity (Wolever *et al.*, 1994; Salmeron *et al.*, 1997a,b). According to the previous findings, a high glycemic load and glycemic index diet may lead to heart disease (Liu *et al.*, 2000; Ford and Liu, 2001; Liu and Manson, 2001) and type 2 diabetes (Salmeron *et al.*, 1997a,b). The glycemic index is a valuable model in diabetes management and weight loss programs. Foods with a low glycemic index generate a more consistent increase in blood sugar and insulin levels due to their slow digestion and absorption and are associated with health benefits such as improved glycemic control in normal and diabetic people (Jenkins *et al.*, 1988).

The edible mushrooms out-perform in terms of nutritional composition, including protein, vitamin content, and medically important components (Thu *et al.*, 2020; Rahman *et al.*, 2021; Ogbole *et al.*, 2021). Furthermore, mushrooms are lower in fat, and higher in dietary fibre, carbs, and protein than grains, legumes, fruits, and vegetables (Ghosh, 1990). Due to their immunoregulatory and therapeutic properties, they have provided health advantages for various disorders, including diabetes, obesity, cardiovascular disease, hyperacidity, constipation, cancer, blood pressure, and hypertension (Ma *et al.*, 2018; Tung *et al.*, 2020). Homoglycans (β-1,3 glucan), heteroglycans, heterogalactans, and heteromannans are the main structural polysaccharides in mushrooms. The polysaccharides mentioned above and terpenes (secondary metabolites) improve insulin resistance and lipid metabolism by inhibiting α-glucosidase, supporting glucose transporter 4 actions, and lowering inflammatory variables (Yang *et al.*, 2019; Dasgupta *et al.*, 2019). Because of their low glycemic index and lack of sugar and carbohydrate, mushrooms are particularly useful in preventing diabetes mellitus. *Lentinula edodes*, a medicinally important mushroom that grows on oak trees, is one of the most widely grown mushrooms, accounting for 25% of the total world mushroom production, accompanied by *Pleurotus ostreatus* (15%) and *A. bisporus* (9.3%) (China Edible Fungi Association, 2017). Chang *et al*., 1996; Miles *et al*., 1997 described this mushroom as having a distinct flavour, aroma, excellent nutritional content, and medicinal characteristics. However, the glycemic index and glycemic load connected to *L. edodes* mushrooms are not well understood. This study aimed to determine the glycemic index and glycemic load of different strains of *L. edodes* mushroom in an S.D. rat model to develop functional foods with a low glycemic index that could be used as a potential anti-diabetic agent.

MATERIALS AND METHODS

Cultivation of *Lentinula edodes* mushroom

Five strains of *Lentinula edodes* mushroom, DMRO-34, DMRO-35, DMRO-356, DMRO-623, and DMRO-388, were procured from the ICAR-Directorate of Mushroom Research, Solan germplasm collection bank. These strains were grown on sawdust, and the harvested fruit bodies were thoroughly dried inside a 40°C oven until crisp. Dried mushrooms were ground into powder using a mechanical grinder.

Experimental rats and housing condition

This study used 36 healthy young adult male S.D. (Sprague Dawley) rats (aged eight weeks and 150-250 g body weight). Each cage held six rats (n=6). Rats were randomly divided into groups and housed in different cages to eliminate bias based on their body weight. These rats were grown accustomed to the experimental setup for 5-7 days before receiving the mushroom dose. The rats were kept in polypropylene cages with stainless steel roof grills at 22°C, with 12 hours of light and darkness alternated and 30-70% relative humidity. After washing, a clean paddy husk was used as bedding in these cages. The rats had unrestricted access to pelleted food and water. The rats were weighed and recorded and dieted for 12 hours after a six-day acclimation period. The Indian Institute of Toxicology Research approved the experimental protocol (Approval Number: IAEC/VVB/MAS/1819/011).

Glycemic index of various *L. edodes* mushroom strains in Sprague Dawley rats

Thirty rats served as the experimental group (GII-V), while six served as the control group (G I). Following a 24-hour fast to accustom rats, six rats from each of the five experimental groups were given 2000 mg of one of five mushroom strains, with the remaining 24 rats receiving rest strains. The rats in the control group were given only 2000 mg of glucose. The blood glucose levels were measured in the blood sample withdrawn from the tail vein at time zero (before dosing), and 15, 30, 45, 60, 90, and 120 minutes after the consumption of glucose and the different strains of mushroom in all rats from each group.

Blood glucose response measurement

Blood samples were drawn from rats of all groups at each time interval. Curves of blood glucose were created using values of blood glucose obtained from rats at times 0, 15, 30, 60, and 120 minutes after consuming glucose and mushroom from each group. The Incremental Area Under the Curve (IAUC) was measured using the trapezoidal rule (Gibaldi and Perrier, 1982) for each rat within each group separately. The curves were obtained from rats treated with reference food (glucose) and with the various test strains of the mushroom. IAUC is the total value obtained from the trapezoids between the curve of blood glucose and the horizontal baseline parallel to the x-axis from time 0 to time 120 min to represent the overall increase in blood glucose level after each group was treated with reference glucose or different strains of the mushroom. The glycemic index (GI) of mushroom was measured using Incremental Area Under two hours of the curve of blood glucose (IAUC) for the test item used, i.e., mushroom, divided by IAUC for standard glucose solution as per the procedure of Jenkins *et al*. (1981); Wolever and Jenkins (1986) and Wolever *et al*. (1991) which were also reported by FAO/WHO (1997) using the following equation:

$$GL= \frac{\text{Incremental area under 2 hr of the curve of blood glucose for mushroom}}{\text{Incremental area under 2 hr of the curve of blood glucose for glucose}} \times 100$$

Glycemic load (GL)

The Salmeron *et al*., 1997a,b formula was used to determine the Glycemic Load (GL) of different strains of each group. This was obtained by multiplying the percentage of carbohydrate content by the glycemic index. The formula shown below has been used:

$$GL= \frac{\text{Total Carbohydrate (g)}}{100} \times GI$$

Statistical analysis

The glycemic index and glycemic load of *L. edodes* mushroom DMRO-34, DMRO-35, DMRO-356, DMRO-623, and DMRO-388, were calculated as a mean±standard deviation. The data were compared to the controls, and a statistically significant difference was determined by calculating the $p<0.05$ value.

RESULTS

In the current study, all mean values of blood glucose level in 5 mushroom strains of Shiitake and control at different time intervals were determined, and their comparative graph are shown in Fig. 1. Results demonstrated that the maximum blood glucose response was found at 30-minute time intervals in strains DMRO- 356, 623, 388, and 34, respectively, and a high blood glucose response at the 45-minute interval in the strain DMRO-35. When comparing tested strains of blood glucose response with standard blood glucose response, the release pattern of blood glucose response was found fast in strains DMRO-623 and 35, whereas the slowest response was found in strain DMRO-356, then in DMRO-388 and 34, respectively. The sum of the incremental area of standard glucose was found at 175.5, whereas in the test item, the sum of the total incremental area was found at 25.9, 27.9, 23.2, 30.1 and 23.3 mmole/L in strain DMRO-34, 35, 356, 623 and 388, respectively. The lowest GI values (13.2%) were found in strain DMRO-356, and strain DMRO-388 (13.3%), followed by strain DMRO-34 (14.8%), DMRO-35 (15.9%), and DMRO-623 (17.2%); all values fall under the low GI category. Among all tested strains, GI values were better in strains DMRO-356, 388, and 34 than in strains DMRO-35 and 623. In a nutshell, the strain DMRO-356 had the lowest GI value, indicating that the glucose release pattern in the blood/body system is slow. This could help avoid diabetes and obesity and improve cardiovascular problems.

The GI values of the different strains of *L.edodes* mushroom did not differ significantly (>0.05), but they

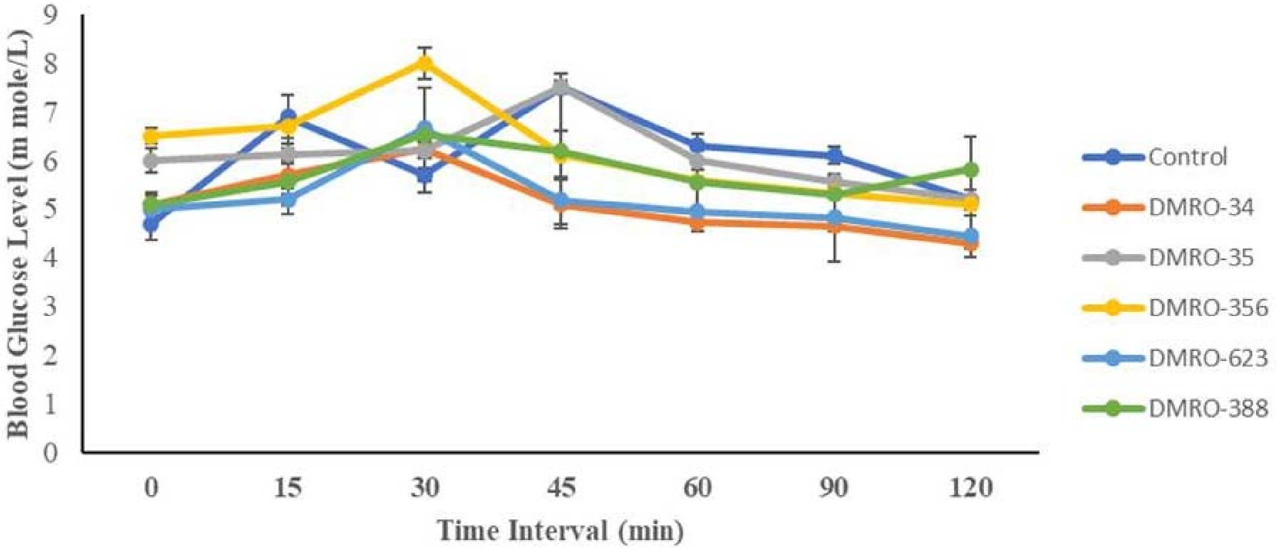

Fig. 1. Blood Glucose Response (GI) of DMRO-34, DMRO-35, DMRO-356, DMRO-623, and DMRO-388 strains compared to control (standard glucose). All values are the mean ± SD of six samples from glucose and each strain

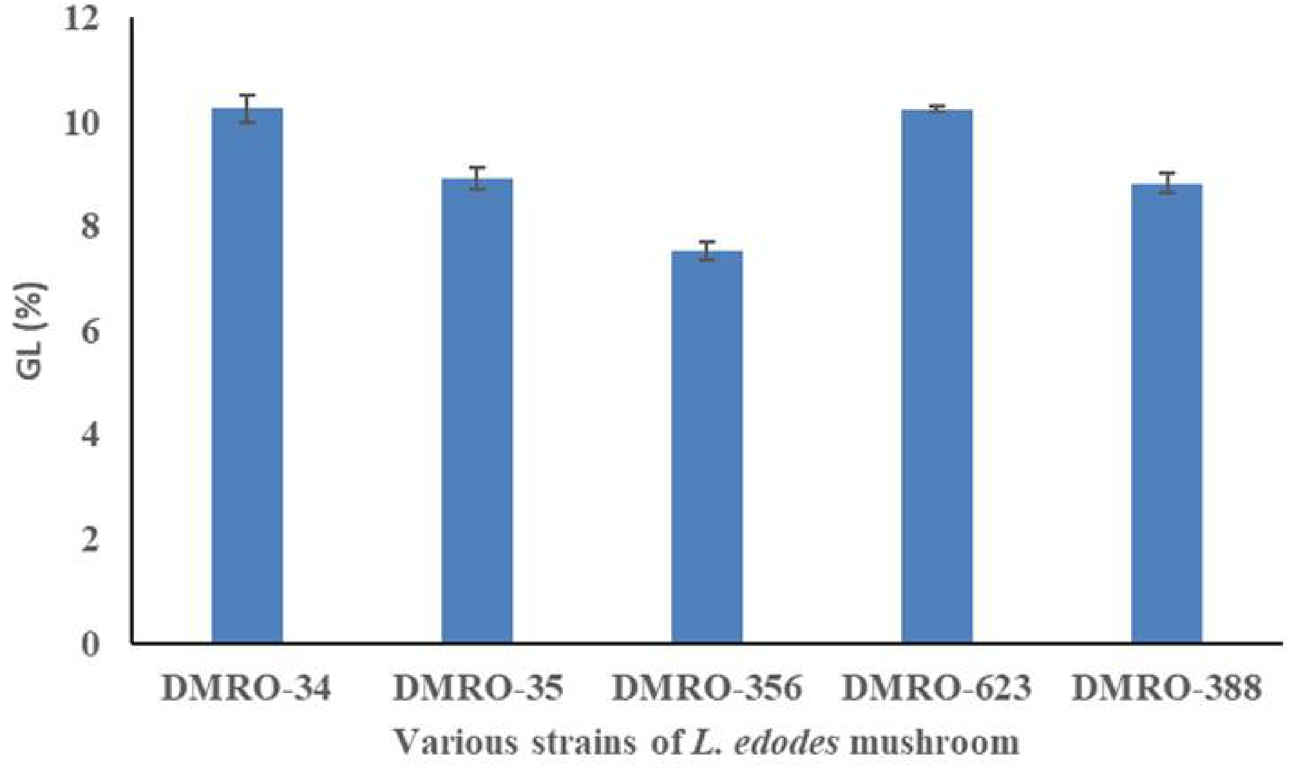

Fig. 2. Status of Carbohydrate content (%) and Glycemic Load (%) among five strains of *L. edodes* mushroom. All values are the mean ± SD of the triplicate of each strain

were significantly (<0.05) lower when compared to the reference value (Glucose). The glycemic load (GL) of the various strains of the mushroom revealed that the strain DMRO-356 had the lowest GL value (7.53%), while strains DMRO-34 and DMRO-623 had the highest GL values (10.25%) (Fig. 2).

DISCUSSION

USDA Food Composition Database, the Federal Ministry of Food and Agriculture of Germany (Bundeslebensmittelschlussel 2019), and the National Institutes of Health have stated the glycemic index and glycemic load of the following mushrooms (Lowcarbcheck, 2022):

Mushrooms	Glycemic index (%)	Glycemic load
Chanterelle	15	0 in a portion of 100 g
Boletus	15	0.2 in a portion of 150 g
Truffles	15	0.1 in a portion of 5g
Oyster	15	0 in a portion of 150g
Morel	15	0 in a portion of 100g

However, there is no comprehensive scientific understanding of the glycemic index and glycemic load concerning *L. edodes* mushrooms. The goal of this study was to determine GI and GL among different *L. edodes* mushroom strains. The glycemic index is obtained by dividing the incremental blood glucose area under the curve after treatment by the test mushroom strains to the relative area after administration with a reference food (Bjorck *et al.*, 2000). Its classification scheme aims to classify foods depending on their blood glucose response (Jenkins *et al*., 1981). In the present study, the glycemic index of the mushroom strains of the same species varied widely from 13.2% in the DMRO-356 to 17.2% in the DMRO-623 (Fig. 1). The GI values of the mushrooms did not change significantly (p>0.05), but they were found considerably (p<0.05) lower in comparison to the reference food (Glucose) at different time intervals. The mushroom's glycemic load (GL) revealed that the strain DMRO-356 had the lowest GL value (7.52%), while strains DMRO-34 and DMRO-623 had the highest GL values (10.26%) (Fig. 2). The findings of this study were consistent with those of Steve (2015), who discovered low GI and GL values when moringa leaves were added to food. In terms of nutrition, the test food's GI and GL should be lower than 55% and 10%, respectively. It is well established, for example, that vegetable-based foods have a lower glycemic index than non-vegetable-based foods (Du *et al.*, 2008). Several factors, including the digestion and absorption rate (FAO/WHO report, 1997; Liljeberg and Bjorck, 1998; Jenkins *et al.*, 2002), the nature of the carbohydrate, and the processing of food, have been shown to affect food's glycemic index in humans.

Foods' GI can be divided into three groups: GI>70% considered as high, GI=56-69% regarded as medium, and GI <55% considered as low, based on the quickness of blood sugar levels rise (Mendosa, 2000), which further depends on the digestion and absorption of accessible sugars and starches in that food (FAO/UN, 1998). High GI foods digest quickly and raise blood sugar, while low GI foods release glucose slowly but steadily. The primary health benefit of low glycemic index and glycemic load foods seems to be that they cause a slower rise in glucose level due to slower stomach emptying, digestion of sugar in the gut lumen, and its absorption into the portal and systemic circulation (Jenkins *et al*., 1981; Wolever *et al*., 1991). Recent research has linked high glycemic load and glycemic index of foods with increased risk of heart disease and diabetes (Liu *et al.*, 2000; Ford and Liu, 2001; Liu and Manson, 2001; Salmeron *et al.*, 1997a,b). As a result, estimation of GI and GL values for different foods may help in calculating carbohydrate-based food content for people with diabetes (FAO/UN, 1998).

CONCLUSION

The glycemic index and glycemic load of *Lentinula edodes* mushroom were not available.

Therefore, the study looked at the glycemic index and glycemic load of various strains of *L. edodes* mushrooms. The mushroom strains studied in the present work were found to fall into the low GI category (0-55%) and glycemic load (0-9%). However, among the five different strains studied, the GI and GL values of strain DMRO-356 (13.22% & 7.53%) and DMRO-388 (13.28% & 8.82%) were found to be lower. The low GI and GL values have a better response for glucose absorption in the blood sample, which may be helpful in diabetic patients and may help prevent human diseases such as cardiac problems.

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