Reishi Mushroom (*Ganoderma lucidum*) Intervention Improves Lipids Profile and Paraoxonase/Arylesterase Activities in Serum as well as Enhances Haemostatic Effects in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Several strategies have been proposed to improve diabetes mellitus (DM) complications, because early treatment and prevention play a pivotal role in reducing the population burden of this disease. The benefits of pharmaceutical agents for treating the disease have long been recommended, but medications may have unwanted side effects. Therefore, the present study was carried out to investigate the potential effects of *Ganoderma lucidum* ethanol extract (GLE) intervention in modulating the hyperglycemia, serum lipids profile and serum paraoxonase/arylesterase activities, and haemostatic effects in streptozotocin-induced diabetic rats. Thirty-six male adult male Sprague-Dawley rats were randomly divided into equal six groups. Group 1: Normal control, normal rats feed with basal diet (BD); Group 2: Model control, diabetic rats feed with BD without intervention; Groups 3-6: GLE, diabetic rats with BD, intervention groups utilizing GLE of 200, 400, 600 and 800 mg/kg bw by oral gavages for 28 consecutive days. Type 2 diabetic rats were obtained by Streptozotocin (STZ) injection. Treatment of rats with streptozotocin caused a significant increased ($p \leq 0.05$) in serum glucose, triglycerides (TG), total cholesterol (TC) and reactive oxygen species (ROS) concentrations by the ratio of 200.13, 554.05, 71.97 and 30.62% compared to normal controls, respectively. The opposite direction was recorded for the high-density lipoprotein cholesterol (HDL-c) and antioxidant enzymes paraoxonase/arylesterase activities which decreased by the ratio of -57.95%, -50.81 and -41.32% compared to normal controls, respectively. Additionally, haemostatic effects (bleeding and clotting times) were increased in different periods of times (7, 14, 21 and 28 days). GLE intervention greatly ameliorated the hyperglycemia, serum lipid profiles, ROS and haemostatic effects in diabetic rats. The rate of amelioration(s) was exhibited a dose- dependent increase with GLE intervention. In conclusion, data of this study provided a basis for the use of GLE for the prevention and/or treatment of type-2 Diabetes mellitus complications such as hyperglycemia, oxidative stress and blood bleeding.

Keywords: Body weight, reactive oxygen species, antioxidant enzymes, bleeding time, clotting time.

INTRODUCTION

Diabetes mellitus (DM) is a group of symptoms defined by inadequate secretion of insulin, incorrect insulin utilization, or a combination of both.. When any of these occur, the body cannot transport sugar from the blood into the cells (Wolff and Dean, 1987). This leads to high blood sugar levels i.e. hyperglycemia. DM is characterized by classical symptoms such classical symptoms such as polydipsia, polyuria, and polyphagia and weight loss. As the disease progresses, to its later stages, several serious complications such as hyperglycemic, cardiomyopathy, neuropathy, ketoacidosis, nephropathy, atherosclerosis etc., can develop (Zatalia and Sanusi, 2013; Elsemelawy et al., 2021; Elhassaneen et al., 2022). In addition, diabetes mellitus generates an excess of reactive oxygen species production (ROS), which cannot be adequately neutralized by antioxidants, resulting in oxidative stress. This oxidative stress leads to damage to the various components of the cell, including the cell membrane, cell organelles (mitochondria and lysosomes), protein and lipid molecules, nucleic acids as well as the vessel wall (Elhassaneen et al., 2022). Several studies have found that oxidative stress plays a role in developing severe degenerative diseases such as atherosclerosis and cardiovascular disease (CVD) (Mart´ın-Gall´an et al., 2003; Mishra and Singh, 2013; Elhassaneen et al., 2021 a,b and c).

Due to the danger of ROS, the aerobic cells in their normal conditions possess defense systems to control the flow of these active radicals, which is known as antioxidant systems. Paraoxonase (PON1) is one of numerous antioxidant enzymes present in intracellular antioxidant systems, which also include non-enzymatic components such as vitamin A, vitamin C, vitamin E, and the enzymes superoxide dismutase, glutathione peroxidase, and catalase (Elhassaneen, 2004, Elhassaneen and El-Badawy,2013; Zatalia and Sanusi, 2013; Elhassanen et al., 2021c); glutathione; vitamins A, C, and E; and the other antioxidants. PON1 prevents

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oxidation (Mackness et al., 1997; Spady, 1999; Mackness and Mackness, 2013), a critical stage in the development of atherosclerosis, in lipoproteins, including HDL and LDL. When PON1 comes into contact with HDL, it forms a binding complex. Paraoxonase and arylesterase activities are present in PON1 due to its ability to hydrolyze organophosphates (containing paraoxon) and aromatic esters (Patel et al., 1990; Sibel et al., 2014). (ARE). When oxidative stress is high, as in diabetes, cardiovascular disease, and dyslipidemia, PON1 and ARE enzyme levels and/or activity rise. However, PON1 and ARE enzyme levels have been observed to decrease proportionally to ROS attack (Boemi et al., 2001; Gbandjaba et al., 2012). Antioxidant therapy has been proposed by some writers as a means of halting PON1 inactivation and/or reduction owing to ROS and so alleviating diabetes and its complications. In conditions of high oxidative stress, such as diabetes, coronary artery disease, and serum lipid profile, PON1 and ARE enzyme activity is reduced, as shown by a number of studies (Boemi et al., 2001; Gbandjaba et al., 2012). Since oxidative stress may lead to PON1's inactivation and/or reduction, antioxidants may help alleviate diabetes and its complications (Bub et al., 2005; Calla and Lynch, 2006; Sibel et al., 2014).

However, CVD continues to be the primary cause of death and disability for persons with diabetes mellitus, accounting for about 80% of all deaths (Kaur et al., 2018). Multiple studies (Preston, 1982; Carr, 2001; Jabeen et al., 2013) point to a role for hemostasis in the onset of vascular complications due to this metabolic disorder. The process of hemostasis is defined as the body's attempt to stop bleeding in an orderly manner. It indicates a sophisticated equilibrium between pro-coagulant, anti-coagulant, and fibrinolytic mechanisms (Lewis and Decie, 2002; Kaur et al., 2018). Platelets, coagulation factors, coagulation inhibitors, fibrinolysis, and blood vessels comprise the most important components of hemostatic system (Lewis and Decie, 2002). Since hyperglycemia promotes platelet-vascular activation and postprandial coagulation activation, it is associated with a hemostatic system. High blood sugar levels, or hyperglycemia, are typical with diabetes mellitus. The severity of microvascular and macrovascular complications is inversely proportional to the degree to which glucose levels are controlled (Preston, 1982; Kluft, 1994; Jabeen et al., 2013). Platelet dysfunction, or hemostasis, was also mentioned by Schneider (2009), who highlighted that both insulin resistance and insulin insufficiency lead to the same thing. Therefore, studying chemicals that aid hemostasis is of medical importance, since it is a potentially life-saving procedure.

Multiple treatment approaches, such as the use of plant components, have been presented during the last few decades in an effort to ameliorate diabetes-related problems, such as oxidative stress and the haemostatic system (vegetables, fruits, herbs, spices, algae etc.). Pharmaceutical formulas (synthetic medications) are notorious for their unpleasant side effects, particularly with extended usage, and their prohibitively costly prices, putting them out of reach for many on a tight budget. This therapeutic strategy has been utilised to circumvent these issues (Jevas, 2011). Our prior research demonstrated that plants are significant suppliers of several bioactive chemicals. They are an intriguing and mostly untapped source for the development of novel diabetes medicines (Sayed Ahmed et al., 2016, El-Nassag et al., 2019; Elhassaneen et al., 2020; 2021a,c).

Reishi mushroom (Ganoderma lucidum), a member of the polyporales family Ganodermaceae, is a prominent medicinal fungus used in traditional Asian medicine. In China, it has been commonly utilised for thousands of years to enhance health and lifespan (Wasser, 2005). Numerous studies have shown that triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins/peptides, and trace minerals are all found in Ganoderma lucidum. (Stojkovic et al., 2014; Elsemelawy et al., 2021; Gharib et al., 2022). Due to its unique bioactive ingredient composition, it has been used for centuries as a folk medicine to cure a variety of human ailments, including cancer, hypertension, ageing, hepatitis, nephritis, and immunodeficiency, among others (McKenna et al., 2002; Wasser, 2005; Sayed-Ahmed et al., 2020). Few studies have examined the link between G. Lucidum intervention and the management of diabetes, as far as we are aware. As such, the study's objective is to determine whether or whether administering Ganoderma lucidum ethanol extract (GLE) to streptozotocin-induced diabetic rats improves their lipid profiles, serum paraoxonase/arylesterase activities, and hemostatic effects.

MATERIAL AND METHODS

1. Materials

1.1. Reishi mushroom

Reishi mushroom (*Ganoderma lucidum*) dried fruits were obtained from ElMisryia Company for Trading Herbs and Medical Plants (Haraz), Cairo, Egypt. Taxonomic confirmation of the mushroom plant part has done by the scientists of Agricultural Plant Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt.

1.2. Chemicals

Streptozotocin, *p*-nitrophenol, phenylacetate and *tris*-HCl were obtained from Sigma Chemical Co., St. Louis, Mo. Casein, as main source of protein was purchased from Morgan Company for Chemicals. Cairo, Egypt. Vitamins and salts mixtures, organic solvents and other chemicals in analytical grade were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

1.3. Kit's

Kit's assays for serum glucose and lipid profile (triglycerides, cholesterol and high-density lipoprotein) were purchased from BIODIAGNOSTIC, Dokki; serum creatinine and urea from Biocon Company, Cairo, Egypt.

Machines: UV-visible-light spectrophotometer (UV-160A; Shimadzu Corporation, Kyoto, Japan) was used for all biochemical analysis.

2. Methods

2.1 Preparation of *Ganoderma lucidum* ethanol extracts (GLE)

A miller (Moulinex Egypt, Al-Araby Co., Egypt) was used to reduce the dried fruits of *Ganoderma lucidum* to a powder, and this powder was then sieved (20 mesh/inch^2) and tested for purity. The approach for making GLE was taken from Oludemi et al. (2017). In summary, ethanol (80%) was used to extract 5 grammes of mushroom powder in a soxhelt semiautomatic apparatus (Velp Co., Italy) for 4-5 hours (20 ± 4 minutes each cycle). A dry solvent extract was then obtained by evaporating the solvent in a rotary evaporator (Büchi R-210, Switzerland) and kept at 4^{0}C until use. Under these circumstances, the total GLE yield in terms of Ganoderma lucidum fruiting bodies was 1.24% (w/w).

2.2. Biological experimental

2.2.1. Ethical approval

All of the biological experiments in this study were approved by the Scientific Research Ethics Committee (Animal Care and Use) at the Faculty of Home Economics at Menoufia University in Shebin El-Kom, Egypt. (approval # 06-SREC-12-2020).

2.2.2. Animals

Adult male albino rats (Sprague-Dewaly) weighing (137±8.35 g per each) were obtained from the Ministry of Health and Population's Helwan Station in Cairo, Egypt.

2.2.3. Basal Diet

The basal diet for the experimental rats feeding was formulated according to the formula provided by AIN (1993) as follows: 10% protein; 10% corn oil; 1% vitamin mixture; 4% mineral mixture; 0.2% choline chloride; 0.3% methionine; 5% cellulose; and 69.5% corn starch. The utilized vitamin and salt combination were formulated according to AIN (1993).

2.2.4. Diabetes induction

According to Sibel et al. (2014), diabetic conditions were induced in normal rats by administering a single intraperitoneal injection of STZ (65 mg/kg body weight, freshly dissolved in 0.05 M sodium citrate buffer, pH 4.5). Blood sugar levels were checked using a Glucostix strip and a glucometer 48 hours after an STZ injection was given via the tail vein (Abbott Glucometer Medicines Products, USA). Diabetic rats were defined as having blood glucose levels of 11 mmol/L (198 mg/dL). After receiving an STZ injection, rats were given a 5% glucose solution diet for 24 hours to prevent catastrophic hypoglycemia caused by enormous insulin release.

2.2.5. Experimental design

The National Research Council's (NRC) regulations (NRC, 1996) were followed for all biological research. The rats (n=36) were kept in a room kept at 24 ± 3 ^{0}C, in wire cages, and in ideal circumstances. For the first week leading up to the experiment, rats were given a standard diet to ensure they were well adapted. After one week, rats were divided into two groups: the first, a normal control group (Group 1, 6 rats), which was still fed a basal diet (BD), and the second, a diabetes induction group (30 rats), which was divided into five subgroups as follows: group (2), model control, fed on standard diet only as a positive control (rats with diabetes) and groups (3-6) fed on basal diet and administered by oral gavages, using a feeding needle with 200, 400, 600, and 800 mg/kg bw *Ganoderma lucidum* ethanol extract (GLE), respectively. The current study's GLE extract concentrations were chosen based on our prior work (Elhassaneen et al., 2016; Sayed Ahmed et al., 2020). All previous groups were kept, each group in one cage, for 28 days. Rats in all groups were weighted at the beginning of the experiments then every week and by the end of the study.

2.2.6. Biological evaluation

During the 28-day study period, the researchers recorded the daily food consumption and weekly body weight of rats. Body weight gain (BWG,%), food intake (FI), and food efficiency ratio (FER) were calculated using the formulas presented by Chapman et al. (1959): FER = Grams growth in body weight (g/28 day)/ Grams feed intake (g/28 day), BWG (%) = (Final weight - Initial weight)/ Initial weight100.

2.2.7. Blood sampling

At the conclusion of the studies, following 12 hours of fasting, the abdominal aorta was used to collect blood

samples from rats that had been scarified under ether anaesthesia. According to Drury and Wallington (1980), Samples of blood were centrifuged for 10 minutes at 3000 rpm after being allowed to clot at room temperature. Careful aspiration of serum was followed by careful transfer to sterile glass tubes, followed by freezing at -200 degrees Celsius until analysis.

2.3. Hematological analysis

2.3.1. Serum glucose

The calorimetric determination of glucose in the serum was performed in accordance with Tietz (1976).

2.3.2. Serum lipid profile

Serum triglycerides (TGs), total cholesterol (TC) and HDL-cholesterol were determined by standard laboratory methods of Fossati and Prenape (1982), Richmod (1973), Lopes-Virella et al. (1977), respectively.

2.3.3. Reactive oxygen species (ROS)

The colorimetric approach proposed by Erel (2005) was used to quantify reactive oxygen species (ROS).

2.3.4. Paraoxonase and Arylesterase activities

Paraoxonase activity was measured using Eckerson's method (Eckerson et al., 1983).'s method. It is stated in units per liter of serum and is defined as 1mmol of p-nitrophenol produced per minute under the given conditions. The rise in absorbance at 412 nm and 250 degrees Celsius was used to calculate the paraoxon hydrolysis rate. To determine aryl esterase activity, phenylacetate was used as a substrate, as detailed by Haagen and Brock (1992). It is defined as the rate at which one millimole of phenol is produced per liter of a reaction mixture comprising one millimole of phenylacetate and ninety-nine millimoles of calcium chloride in 9.0 millimoles of Tris-HCl buffer at pH 8.0 and represented in units of Ku per liter.

2.3.5. Hemostatic effects

Bleeding time (BT)

The technique outlined by Ochei and Kolhatkar (2000) was used to measure BT in animals. Briefly, the tails of each animal were cleansed with methylated spirit and kept out of the cages' perforations. Using a disposable lancet, the tip of the tail was rapidly severed, and the stopwatch was started as soon as the bleeding began. Every 15 seconds, the filter paper was applied to the incision until the paper was no longer stained with blood. BT was then determined as the moment when the wound stopped bleeding. Blood type was identified by counting the number of blood spots removed from filter paper. The location was then multiplied by the amount of time it took to remove the blood, which was 30 seconds. The outcome is then multiplied by 60 min.

Clotting time (CT)

The CT in these animals was determined using the method described by Cole (1987). After thoroughly cleaning with methylated alcohol, the animal's tail was severed using a single-use lancet. The tail was quickly put into four 37°C-maintained, 37°C-warmed glass test tubes. A 37 degrees' Celsius water bath was quickly prepared to mimic the temperature within the tubes. The timer began counting down as soon as the blood was inserted into the tubes. When the blood became gelatinous, the ends of the test tubes were clipped off using scissors and the time noted. The average clotting time was calculated by timing the blood as it coagulated in each of the four tubes.

2.3. Statistical Analysis

Means and standard deviations were provided for the data One-way analysis of variance (ANOVA) and Tukey's multiple comparison post hoc tests was used to establish whether or not there was a statistically significant difference between the two groups. Results were compiled using the statistical program MINITAB 12. (Minitab Inc., State College, PA). The significance level was set at P 0.05 (Duncan et al., 1977).

RESULTS AND DISCUSSION

1. The effect of GLE consumption on ethanolic extracts on diabetic rats' body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER).

Data in Table (1) and Figure (1) showed the effect of GLE intervention on BWG, FI and FER of diabetic rats. Such data indicated that diabetic rats induced by STZ was shown to be significantly ($p \leq 0.05$) lower in BWG (-40.20), FI (-28.08), and FER (-22.37) when compared to the normal control group. GLE was added to rats' meals at concentrations of 200, 400, 600, and 800 mg/kg BW for 28 days induced significantly ($p \leq 0.05$) increasing in BWG (-34.40, -29.77, -19.50 and -12.68%), FI (-24.98, -19.23, -17.87 and -13.95%), and FER (-19.74, -17.11, -13.16 and -6.58%) levels compared to the normal control animals, respectively. With GLE intervention, the rate of rise for each of these measures increased dose-dependently. The current study's findings are consistent with those of other researchers who have examined Ganoderma lucidum and other species of algae (Elhassaneen et al., 2019, 2020, 2021e; Sayed-Ahmed et al., 2020; Essa, 2021; Fayez, 2021). G. lucidum fruits contain a wide variety of bioactive compounds, each of which has several biological effects, including a reduction in BWG, FI, and FER (Sayed Ahmed et al., 2020; Gharib et al., 2022).

Table 1. Effect of GLE intervention on BWG, FI and FER of diabetic rats

Groups	BWG (%)	FI (g/day/rat)	FER
Normal control	0.889± 0.054 [a]	10.97± 0.55 [a]	0.076± 0.007 [a]
Model control	0.532± 0.021 [d]	7.89± 0.83 [c]	0.059± 0.011 [b]
GLE intervention (200 mg/kg BW)	0.583± 0.049 [cd]	8.23± 0.65 [b]	0.061± 0.009 [b]
GLE intervention (400 mg/kg BW)	0.624± 0.048 [c]	8.86± 0.48 [b]	0.063± 0.007 [b]
GLE intervention (600 mg/kg BW)	0.716± 0.029 [bc]	9.01± 0.60 [ab]	0.066± 0.008 [ab]
GLE intervention (800 mg/kg BW)	0.776± 0.039[b]	9.44± 0.72 [a]	0.071± 0.008 [a]

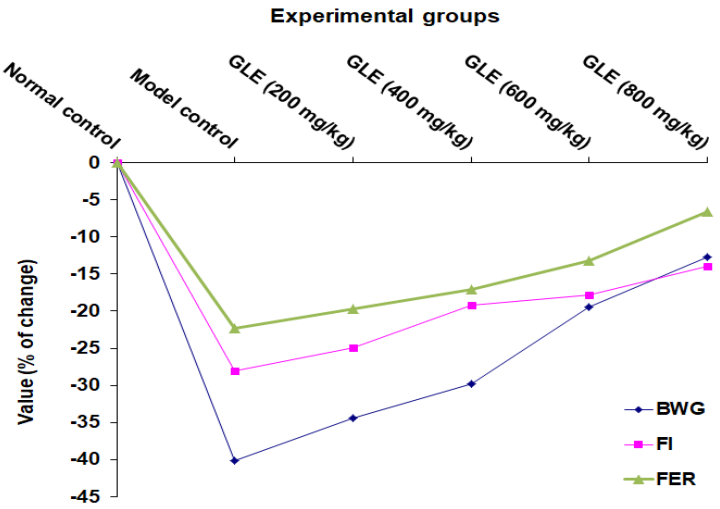

Figure 1. Effect of GLE intervention on BWG, FI and FER (as % of change from the normal control group) of diabetic rats*

* Each value represents the mean of six rats. Details of groups are as shown under Table 1. BWG, body weight gain; FI, feed intake; FER, feed efficiency ratio.

In this direction, several studies reported that injection of animals by CCl_4 induced hepatotoxicity complications including diabetic effects which led to decrease in BWG, FI and FER (Tahoon, 2019, Arafa, 2021 and Elhassaneen et al., 2021d). These abnormalities in BWG, FI, and FER were alleviated by consuming plant components containing bioactive compounds, such as GLE. Also, other studies confirmed that liver disorders in rats induced by diabetes reveal significant ($p \leq 0.05$) decreasing of the BW and FI (Hamzawy et al., 2013; Abd El-Rahman, 2021). Finally, some relevant studies have shown that DM and hepatic diseases can lead to malnutrition, which is one of the main causes of malnutrition in people with these diseases. These malnutrition effects include poor feed intake, maldigestion, malabsorption, and metabolic and nutrient storage abnormalities (Morresion and Hark, 1999; Aly et al., 2017; Abd El-Rahman, 2021; Arafa, 2021).

Results are expressed as means ± SD (n=6). Different superscript letters on the same column indicate significant difference ($P \leq 0.05$). Normal control: healthy rats without intervention; Model control: STZ induced diabetic rats without intervention; GLE intervention: STZ induced diabetic rats with GLE intervention. BWG, body weight gain; FI, feed intake; FER, feed efficiency ratio.

2. The effect of GLE on serum glucose of diabetic rats

The effect of GLE on serum glucose concentration of diabetic rats induced by STZ was shown in Table (2) and Figure (2). Blood glucose levels in STZ-treated rats were significantly ($p \leq 0.01$) higher than in the control group by a ratio of 200.13. Compared to normal control animals, blood glucose content was reduced by 175.65%, 148.63%, 92.12%, and 73.74% after 28 days of GLE intervention at concentrations of 200, 400, 600, and 800 mg/kg BW in the diets of rats. With GLE intervention, the rate of rising blood glucose revealed a dose-dependent rise. In general, STZ is often used to cause diabetes in laboratory animals. This is due to the potential of this substance to destroy the pancreatic islet

-cells, hence inducing chronic or permanent diabetes in these animals ((Mathe, 1995; Elhassaneen et al., 2021c).

The submitted findings demonstrated that diabetic rats had considerably higher blood glucose concentrations than normal rats. Chronic hyperglycemia, like IDDM, can be induced by a lack of insulin secretion (Kandeil et al., 2008). STZ causes diabetes in the same environment by producing reactive oxygen species (ROS) that damage DNA, inducing DNA-strand breaks in -cells (Lenzen, 2008). The activation of poly (ADP-ribose) polymerase (PARP), which uses NAD+ as a substrate, is required for repairing DNA strand breaks. Consequently, intracellular NAD^+ levels decline. Such a decrease in NAD^+ inhibits the energy molecule (ATP) production and cellular processes, such as insulin synthesis and release, resulting in the death of the β-cell (Pusztai et al., 1996).

These interventions would reduce glucose absorption by peripheral tissues, including muscles and adipose tissue (glycogenolysis), while increasing gluconeogenesis and hepatic glucose production (Raju et al., 2001; Beck-Nielsen, 2002 and Jung *et al.*, 2011). These mechanisms are consistent with the findings of the current investigation. In addition, a number of investigations demonstrated that diabetic rats had considerably lower blood insulin levels than normal rats (Kandeil et al., 2008; Elsemelawy et al., 2021). On the other hand, the current investigation revealed that GLE has an effective hypoglycemic impact against diabetes produced by STZ. Such effect(s) may be related to the huge bioactive constituents found in GLE. Several studies reported that *Ganoderma lucidum* and its extracts are a rich in phenolics, lycopene, polysaccharides, Terpenoids, flavonoids, Triterpenoids, sterols, cyclo-octasulfur, an ergosterol peroxide , cerebrosides, vitamins (A, B and E) (McKenna *et al.*, 2002; Gao *et al.*, 2003; Liu *et al.*, 2016; Darija *et al.*, 2018; Gharib et al., 2022). These bioactive constituents are known for various biological properties, including antioxidant activity, inhibition of lipid oxidation and free radical scavenging action, glucose response improvement, and alleviation of metabolic dysregulation of free fatty acids and insulin resistance associated with T2DM, according to our previous studies with others (Elhassaneen et al., 2013 and 2016a; Elmaadawy et al., 2016; Aly et al., 2017; Elbasouny et al., 2019, Elsemelawy et al., 2021; Gharib et al., 2022). Also, the phenolic compound in *Ganoderma lucidum* exhibited high potency of antioxidant activities which lead to inhibit the α-amylase and sucrase, the principle substance for suppressing of the postprandial hyperglycemia (Tiwari and Madhusudana, 2002). Other bioactive compounds in *Ganoderma lucidum*, polysaccharides, have potential hypoglycemic activity in experimental animals (Wasser, 2005).

Table 2. Effect of GLE intervention on serum glucose concentration of diabetic rats

Groups	Serum glucose conc. (Mean ±SD, mg/dL)
Normal control	98.67± 3.67 [e]
Model control	296.14± 12.09 [a]
GLE intervention (200 mg/kg BW)	271.99± 10.54 [a]
GLE intervention (400 mg/kg BW)	245.33± 10.90 [b]
GLE intervention (600 mg/kg BW)	189.56± 7.83 [c]
GLE intervention (800 mg/kg BW)	171.43± 8.12 [d]

Each value represents mean ± SD (n= 6). Different superscript letters on the same column indicate significant difference ($P \leq 0.05$).

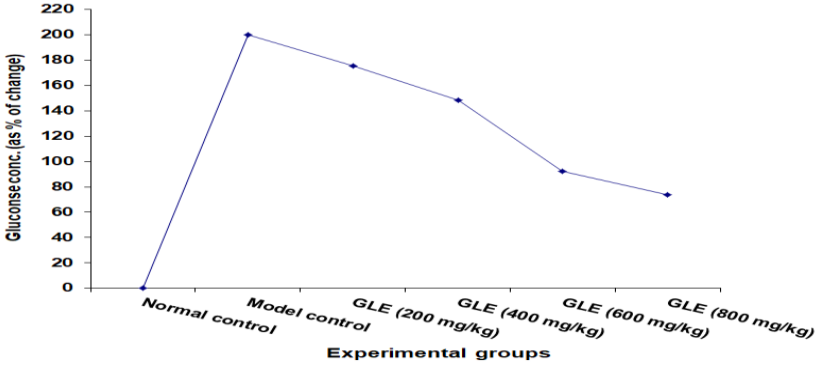

Figure 2. Effect of GLE intervention on serum glucose concentration (as % of change from the normal control group) of diabetic rats*

* Each value represents the mean of six rats.

3. Effect of GLE intervention on serum reactive oxygen species (ROS) concentration of diabetic rats

The state of oxidative stress in diabetic rats treated with GLE was determined by monitoring the blood concentration of reactive oxygen species (ROS) (Table 3 and Figure 3). STZ generated a substantial rise ($p \leq 0.05$) in serum ROS levels by 30.62 percentage points in comparison to normal controls. Intervention with GLE (200, 400, 600 to 800 mg/kg bw/day) in rat diets for 28 days led to significantly ($p \leq 0.05$) decrease the levels of ROS which recorded 25.24, 21.77, 12.20 and 7.41 compared to the normal controls. With GLE intervention, the rate of decrease in serum ROS showed a dose-dependent rise. Similar studies have investigated the byproducts or indicators of oxidative processes mediated by free radicals to identify oxidative stress associated with diabetes (Aly et al., 2017, Abd El-Rahman, 2021, Elsemelawy et al., 2021, Elhassaneen et al., 2016 and 2021e,2022). Thus, ROS is often utilized as a biomarker to provide an acceptable indication of oxidative stress state. The current research revealed that the degree of oxidative stress in diabetic rats was reduced as a result of GLE-induced ROS decrease. Previous research has also shown a substantial correlation between ROS serum concentrations and the pathogenic phases of a number of illnesses, including diabetes, since ROS concentrations increased significantly in diabetes (Elmaadawy et al., 2016; Elsemelawy et al., 2021, Elhassaneen et al., 2021e, 2022). Also, systemic metabolic alterations associated with DM such hyperglycemia, liver and kidney dysfunctions, alteration in serum lipids profile etc. in experimental animals contribute to the increase in oxidative stress (Elsemelawy et al., 2021; Elhassaneen e al., 2016, 2021 and 2022). Maintaining an appropriate level of oxidative stress would be facilitated by the GLE-induced regulation of ROS levels throughout the body. In accordance with this, Elsemelawy *et al.* (2021) revealed that oxidative stress indices were enhanced in diabetic rats and that *Ganoderma lucidum* alcoholic extract had an antioxidant and chemoprotective impact on the liver due to its high bioactive component content. Also, Wasser, (2005) and Gharib *et al.*, (2022) reviewed that *Ganoderma lucidum* may be employed as a dietary natural antioxidant intervention to prevent free radical-related disorders. The antioxidant and free radical scavenging activities of its bioactive components may account for many of its beneficial qualities. Catalase, glutathione peroxidase, and superoxide dismutase are just some of the antioxidant enzymes that are stimulated, and they all work together to keep lipids from oxidising (Wasser, 2005; Elsemelawy et al., 2021; Gharib et al., 2022).

Table 3. Effect of GLE intervention on serum reactive oxygen species (ROS) concentration of diabetic rats

Groups	ROS conc. (Mean ±SD, U/mL)
Normal control	54.96± 3.98 [c]
Model control	71.79± 5.22 [a]
GLE intervention (200 mg/kg b w)	68.83± 4.72 [a]
GLE intervention (400 mg/kg b w)	66.93± 3.22 [a]
GLE intervention (600 mg/kg b w)	61.66± 7.32 [b]
GLE intervention (800 mg/kg b w)	59.03± 5.02 [b]

Each value represents mean ± SD (n = 6). Different superscript letters on the same column indicate significant difference ($P \leq 0.05$).

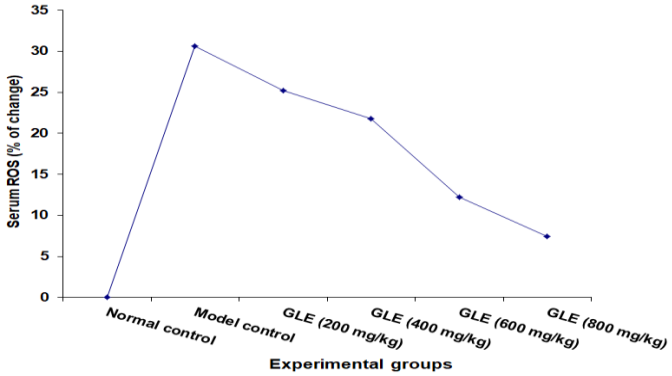

Figure 3. Effect of GLE intervention on serum reactive oxygen species (ROS) concentration (as % of change from the normal control group) of diabetic rats[*]

[*] Each value represents mean of six rats.

4. Effect of GLE intervention on serum lipid profile of diabetic rats

The effect of GLE on serum lipid profiles of diabetic rats was shown in Table (4) and Figure (4). From these data, it was possible to conclude that the administration of STZ to rats resulted in a significant increase ($p \leq 0.05$) in serum triglycerides (TGs) and total cholesterol (TC) by the ratio 554.05 and 71.97% compared to normal controls animals, respectively. The opposite direction was observed for HDL-c level which significantly ($p \leq 0.05$) decreased by -57.95%. Intervention with GLE by the levels of 200, 400, 600 to 800 mg/kg bw/day in rat diets for 24 days to significantly ($p \leq 0.05$) decrease the levels of TGs and TC which recorded 497.30, 435.14, 224.32 and 202.70%, and 71.97, 42.56, 37.02 and 34.26% compared to the normal controls group, respectively. For HDL-c, the rate of increasing was -50.00, -35.76, -13.91 and -9.93% compared to the normal controls group, respectively. In a dose-dependent manner, GLE intervention decreased TGs and TC and increased HDL-c. Thus, data of the present study reported that DM was associated with hyperlipidemic (TGs) and hypercholesterolemic (TC) i.e. increased the serum bad lipid particles (TGs and TC) and decrease the serum good lipid particles (HDL-c) which significantly ($p \leq 0.05$) improved by GLE intervention. Our several previous studies reported the same behavior by different pant parts other than Ganoderma lucidum (Elhassaneen et al., 2021 a,b and c; Elhassaneen et al., 2022; Gharib et al., 2022). In humans, elevated blood lipid (TG) and lipoprotein levels, especially LDL, increase the risk of CVD, fatty liver, carcinogenesis, peripheral vascular disease, and atherosclerosis (Derosa et al., 2015). Several decades ago, many synthetic oral antihyperlipidemic and antihypercholesterolemic drugs were discovered but almost of them have adverse side effects (Heidarian and Rafieian-Kopaei, 2012). Findings of this study confirmed the effectiveness of a *Ganoderma lucidum* in improving the serum lipid profiles resulting from DM induced by STZ without any side effects. Such data were in agree with Gharib et al. (2022) who found that *Ganoderma lucidum* extracts recorded the anti-atherosclerotic effect. In addition, Wasser (2005) noted that Ganoderma lucidum displayed antihyperlipidemic and antihypercholesterolemic benefits by one or more of the following mechanisms: lowered cholesterol absorption, plasma cholesterol level, liver VLDL concentration, and triacylglycerol.

Table 4. Effect of GLE intervention on serum lipid profile of diabetic rats

Groups	Triglycerides (TG, mmol/l)	High density lipoprotein cholesterol (HDL-c, mmol/l)	Total cholesterol (TC, mmol/l)
Normal control	0.30 ± 0.02 ^{c}	3.02 ± 0.07 ^{a}	2.89 ± 0.10 ^{c}
Model control	2.90 ± 0.41 ^{a}	1.27 ± 0.12 ^{d}	4.97 ± 0.45 ^{a}
GLE intervention (200 mg/kg BW)	2.81 ± 0.24 ^{a}	1.51 ± 0.21 ^{c}	4.12 ± 0.39 ^{ab}
GLE intervention (400 mg/kg BW)	2.02 ± 0.14 ^{a}	1.94 ± 0.09 ^{b}	3.96 ± 0.11 ^{b}
GLE intervention (600 mg/kg BW)	1.33 ± 0.24 ^{b}	2.60 ± 0.21 ^{a}	3.88 ± 0.09 ^{b}
GLE intervention (800 mg/kg BW)	1.22 ± 0.31 ^{b}	2.72 ± 0.44 ^{a}	3.53 ±0.27 ^{bc}

Results are expressed as means ± SD (n = 6). Different superscript letters on the same column indicate significant difference ($P \leq 0.05$).

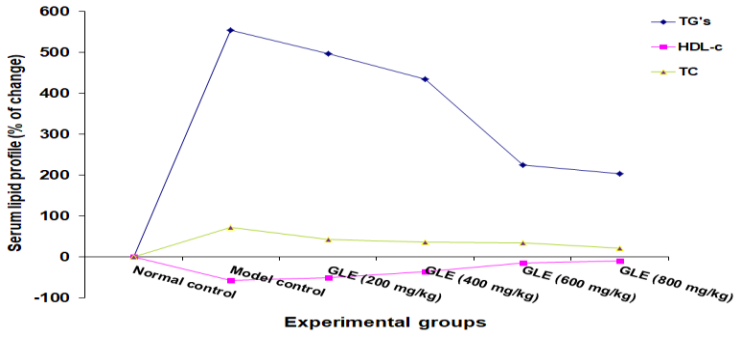

Figure 4. Effect of GLE intervention on serum lipid profile (as % of change from the normal control group) of diabetic rats*

* Each value represents mean of six rats. Details of groups are as shown under Table 1. TG's, triglycerides; HDL-c, high density lipoprotein cholesterol; TC, total cholesterol

In addition, they indicated that the decrease in VLDL level was attributable to a number of mechanisms, such as the decrease in VLDL synthesis and liver secretion, and the suppression of intestinal cholesterol absorption. Ganoderma lucidum's antihyperlipidemic and antihypercholesterolemic effects have been linked to multiple mechanisms, including: enhancing the endogenous transformation of cholesterol to bile acid; encouraging the development of hepatic LDL receptors, which raised plasma LDL clearance; and blockading acyl-CoA as a significant step of lipid metabolism, such as cholesterol acyltransferase (Wasser, 2005; Sayed-Ahmed et al. 2020; Elsemelawy et al., 2021; Gharib et al., 2022). Ganoderma lucidum has been studied for its potential utility in treating CVD including atherosclerosis due to its hypolipidemic and hypocholesterolemic qualities.

5. Effects of GLE intervention on plasma paraoxonase activity of diabetic rats

The effects of GLE intervention on plasma paraoxonase activity of diabetic rats induced by STZ were shown in Table (5) and Figure (5). Serum paraoxonase and arylesterase activity in the diabetes (model) group were significantly ($P \leq 0.01$) reduced by the ratios -50.81 and -41.32%, respectively, as compared to those in the normal control group. Intervention with GLE by the levels of 200, 400, 600 to 800 mg/kg bw/day in rat diets for 28 days significantly ($p \leq 0.05$) elevated the levels of those parameters which recorded -46.74, -43.49, -39.21 and -33.45%, and -38.47, -34.48, -26.93 and -24.88% compared to the normal controls group, respectively.

Similar to previous investigations in diabetic rats and humans (Wegner et al., 2011; Aviram and Vaya, 2013; Sibel et al., 2014), The paraoxonase and arylesterase activity in the serum of STZ-diabetic rats were found to be lower than those in non-diabetic rats. Lower paraoxonase and/or arylesterase activity has been found in people with diabetes (Boemi et al., 2001; Amine et al., 2011; Sibel et al., 2014), which is one of the disorders linked to oxidative stress. A reduction in serum paraoxonase activity in DM may be associated with elevated blood glucose and/or oxidative stress since glycol oxidation of HDL in a hyperglycemic state has been found to impede enzyme activity. Glycosylation and oxidative alteration of transcription factors or DNA also reduce their ability to stimulate protein synthesis in the cell. This trend was also seen while studying arylesterase activity. GLE intervention groups have been shown to have higher levels of serum paraoxonase and arylesterase (PON1) enzyme activity. This may be due to GLE's direct stimulating action on PON1 and/or PON1's biological activities, such as its antioxidant capacity, free radical scavenging activities, and inhibition of lipid peroxidation (Wasser, 2005; Elsemelawy et al., 2021; Gharib et al., 2022). Parallel findings were obtained by Sibel et al. (2014), who used another antioxidant (vitamin B6) to treat diabetic rats, resulting in an increase in the activity of many biological antioxidant enzymes, such as GSH-Px, SOD, and PON1. Other studies have shown that when PON1 attaches to high-density lipoprotein (HDL), it shields lipoproteins such as HDL and LDL from oxidation, which is essential for the development of atherosclerosis (Mackness and Mackness, 2013; Sibel et al., 2014). Therefore, data of the present study suggest that intervention with Ganoderma lucidum can protect PON1 from inactivation and/or diminution arising from oxidative stress, thus improving diabetes and related complications.

Table 5. Effects of GLE intervention on plasma paraoxonase activity of diabetic rats induced

Groups	Paraoxonase activity (Mean ±SD, U/L)	Arylesterase activity (Mean ±SD, kU/L)
Normal control	119.36 ± 1.90 [a]	139.15± 6.11 [a]
Model control	58.71 ± 4.24 [c]	81.66± 7.23 [d]
GLE intervention (200 mg/kg b w)	63.57 ± 2.76 [bc]	85.62± 5.72 [cd]
GLE intervention (400 mg/kg b w)	67.45 ± 2.10 [b]	91.17± 4.82 [c]
GLE intervention (600 mg/kg b w)	72.56 ± 0.77 [b]	101.67± 4.21 [b]
GLE intervention (800 mg/kg b w)	79.43 ± 3.90 [b]	104.53± 6.08 [b]

Results are expressed as means ± SD (n = 6). Different superscript lowercase letters on the same column indicate significant difference ($P \leq 0.05$).

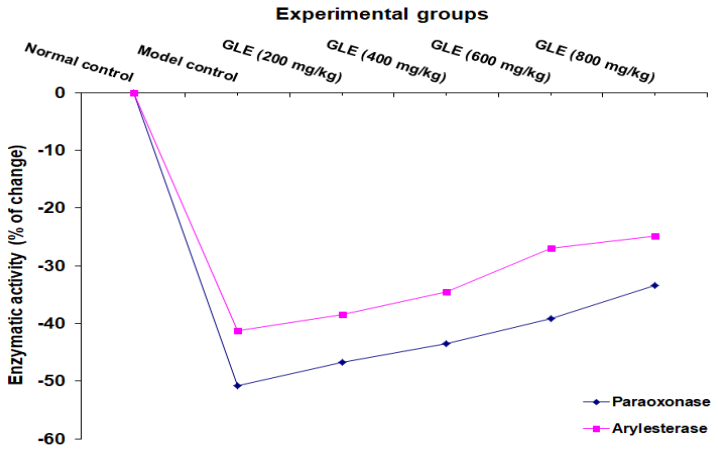

Figure 5. Effects of GLE intervention on plasma paraoxonase activity (as % of change from the normal control group) of diabetic rats

* Each value represents mean of six rats.

6. Effect of GLE intervention on haemostasis process of diabetic rats

The effects of GLE intervention on haemostasis process parameters of diabetic rats induced by STZ were shown in Tables (6 and 7) and Figures (6 and 7). Such data indicated that bleeding and clotting times in the diabetic (model) group were significantly ($p \leq 0.01$) increased by the ratio 9.22, 26.34, 11.73 and 10.04% and 10.10, 9.48, 2.73 and 10.92%, respectively, when compared with those of the normal control group. Intervention with GLE by the levels of 200, 400, 600 to 800 mg/kg bw/day in rat diets for 28 days significantly ($p \leq 0.05$) decreased the values of those parameters by different rates when compared with the normal controls group.

Coagulation and fibrinolysis work together to maintain blood fluidity and prevent hemorrhage from damaged arteries during hemostasis. The subsequent processes include vasoconstriction, activation of thrombin, adhesion and activation of platelets, synthesis of fibrin from circulating fibrinogen, and inactivation of the coagulation cascade (Dapper et al., 2007; Tanko et al., 2012). This research was conducted to learn how Ganoderma lucidum impacts hemostasis and, more specifically, how it affects bleeding and clotting times. Analysis of bleeding and clotting times provides insight into the many coagulation components of the intrinsic route (Dapper et al., 2007; Tanko et al., 2012). The plasma fibrinogen, for instance, has been shown to expedite the production of fibrin polymers, resulting in more efficient clot formation. (Guyton and Hall, 2000). Consequently, deficiencies in these intrinsic pathway variables will have an effect on the outcomes. The current investigation revealed that the GLE had a substantially (p0.05) shortened clotting time, indicating a rise in several clotting components implicated in the intrinsic route. These findings are consistent with those reported by Tanko et al. (2012), who discovered that an aqueous extract of Ganoderma lucidum increased the hemostatic activities, i.e. bleeding and clotting times, in normal rats. Other plant parts such as *Aspilia africana* and *Ageratum conyzoides* exhibited the same effects (Okoli et al., 2007; Bamidele et al., 2010).

Table 6. Effect of GLE intervention on bleeding time of diabetic rats

Groups	Bleeding time (Minutes)			
	Day 7	Day 14	Day 21	Day 28
Normal control	5.93± 0.35 ^{ab}	4.10± 0.32 ^{b}	3.98± 0.24 ^{b}	3.65± 0.52 ^{c}
Model control	6.48± 0.38 ^{a}	5.18± 0.46 ^{a}	4.45± 0.39 ^{ab}	4.02± 0.79 ^{b}
GLE intervention (200 mg/kg BW)	5.27± 0.12 ^{b}	5.22± 0.22 ^{a}	5.02± 0.28 ^{a}	5.21± 0.46 ^{a}
GLE intervention (400 mg/kg BW)	5.03± 0.31 ^{bc}	4.75± 0.19 ^{b}	5.25± 0.17 ^{a}	4.42± 0.37 ^{b}
GLE intervention (600 mg/kg BW)	5.30± 0.20 ^{b}	4.94± 0.42 ^{ab}	4.44± 0.45 ^{ab}	4.21± 0.61 ^{b}
GLE intervention (800 mg/kg BW)	4.96± 0.26 ^{c}	5.08± 0.35 ^{a}	3.26± 0.24 ^{c}	3.30± 0.39 ^{c}

Results are expressed as means ± SD (n = 6). Different superscript letters on the same column indicate significant difference ($P \leq 0.05$).

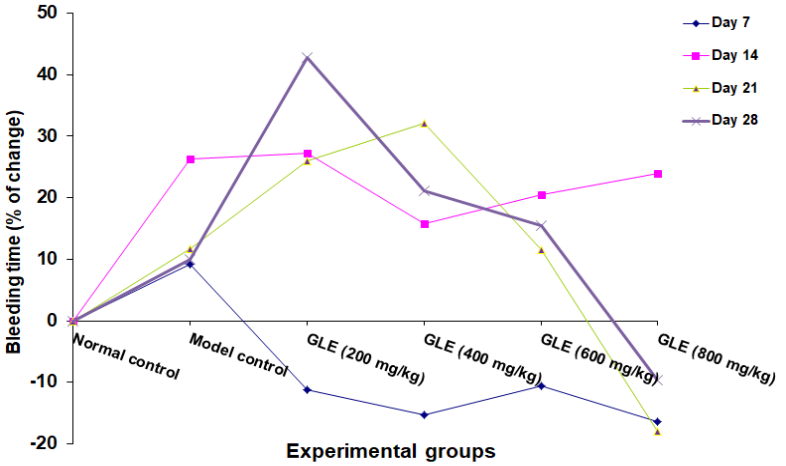

Figure 6. Effect of GLE intervention on bleeding time (as % of change from the normal control group) of diabetic rats*

* Each value represents mean of six rats.

Table 7. Effect of GLE intervention on clotting time of diabetic rats

Groups	Clotting time (Minutes)			
	Day 7	Day 14	Day 21	Day 28
Normal control	7.69± 0.18 ^{a}	6.87± 0.19 ^{a}	5.87± 0.23 ^{a}	4.67± 0.18 ^{ab}
Model control	8.46± 0.20 ^{a}	7.52± 0.71 ^{a}	6.03± 0.28 ^{a}	5.18± 0.58 ^{a}
GLE intervention (200 mg/kg BW)	7.04± 0.24 ^{ab}	5.61± 0.31 ^{ab}	4.56± 0.64 ^{b}	4.70± 0.29 ^{a}
GLE intervention (400 mg/kg BW)	5.23± 0.25 ^{c}	4.94± 0.32 ^{c}	4.41± 0.36 ^{b}	3.89± 0.44 ^{b}
GLE intervention (600 mg/kg BW)	6.28± 0.44 ^{b}	6.30± 0.38 ^{a}	5.14± 0.33 ^{a}	5.04± 0.38 ^{a}
GLE intervention (800 mg/kg BW)	6.11± 0.32 ^{b}	5.24± 0.24 ^{b}	3.88± 0.50 ^{c}	3.73± 0.26 ^{b}

Results are expressed as means ± SD (n = 6). Different superscript letters on the same column indicate significant difference ($P \leq 0.05$).

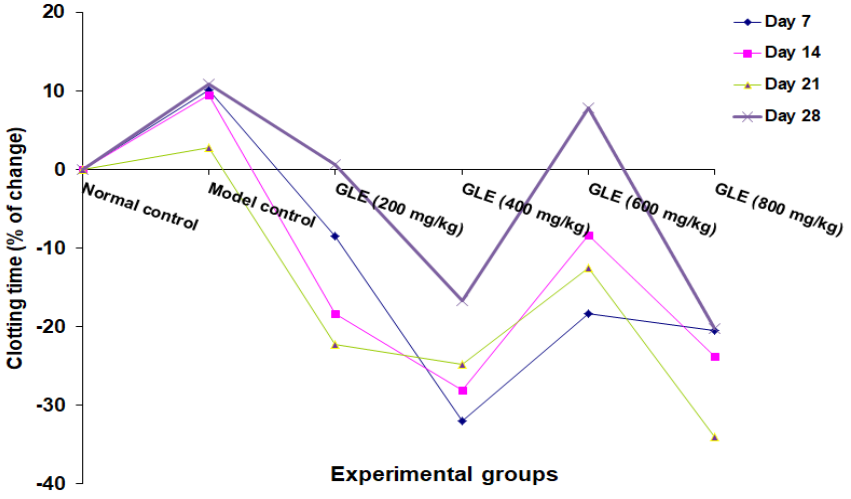

Figure 7. Effect of GLE intervention on clotting time (as % of change from the normal control group) of diabetic rats.

* Each value represents mean of six rats.

Gharib et al. (2022) 's phytochemical composition of Ganoderma lucidum revealed a high content of bioactive compounds such as polysaccharides, polyphenols, flavonoids, tannins, alkaloids, and lycopene, which exhibited many biologically roles such as antioxidant activity, scavenging of free radicals, and inhibition of lipid oxidation. Several studies have shown that tannins and polyphenols have a function in plant hemostasis by stopping bleeding from injured or wounded arteries by precipitating proteins to form vascular plugs. (Okoli et al., 2007; Bamidele et al., 2010). Therefore, it is probable that the haemostatic mechanism of Ganoderma lucidum is associated with the presence of these phytochemicals and their biological effects.

CONCLUSION

The results of the current investigation revealed that *Ganoderma lucidum* ethanol extract (GLE) may partly relieve diabetic complications in rats caused by streptozotocin (STZ). In diabetic rats, complications include decreased hyperglycemia, increased serum antioxidant enzyme (paraoxonase and arylesterase) activity, and improved haemostatic effects. These ameliorative effects may result from GLE's high concentration of bioactive compounds and their physiologically active actions, such as its ability to serve as an antioxidant and remove free radicals from the body. Such phytochemicals and their biological activity may also be associated with GLE's hemostatic mechanism (stop bleeding from damaged or wounded arteries). These findings show that elevated blood glucose and other complications of T2DM can be avoided or managed with GLE and bleeding.

CONFLICT OF INTERESTS

Regarding the publishing of this article, the authors state that no conflicts of interest exist.

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الملخص العربي

التدخل الغذائى بالفطر الريشي (جانوديرما لوسيدوم) يعمل على تحسين مستوى الدهون وأنشطة انزيمات باروكسوناز/ أريلستيراز في الدم إضافة إلى تعزيز تأثيرات تخثر الدم في الفئران المصابة بمرض السكري المستحث بالاستربتوزوتوسين

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تم اقتراح العديد من الاستراتيجيات لتحسين مضاعفات مرض السكري، حيث أن العلاج المبكر والوقاية يلعبان دورًا محوريًا في تقليل العبء السكاني لهذا المرض. لطالما تمت التوصية بفوائد المستحضرات الصيدلانية المخلقة لعلاج المرض، الا ان اغلب تلك الحضرات يكون لها آثار جانبية غير مرغوب فيها. لذلك، أجريت الدراسة الحالية للتحقيق في الآثار المحتملة للتدخل الغذائى بالمستخلص الايثانولى للفطر الريشى (جانوديرما لوسيدوم) (GLE) في تعديل ارتفاع السكر وصورة الدهون وأنشطة الانزيمات المضادة للاكسدة (باروكسوناز/ أريلستيراز) في الدم وكذلك تأثيرات تخثر الدم في الفئران المصابة بداء السكري المستحث بالاستربتوزوتوسين. وللتحقيق من ذلك تم تقسيم ستة وثلاثون من ذكور الفئران سبراج داولي بشكل عشوائي إلى ست مجموعات متساوية. المجموعة ١: المجموعة الضابطة الطبيعية، وفيها تتغذى الفئران على النظام الغذائي الأساسي (BD)؛ المجموعة ٢: المجموعة الضابطة الموجبة، وفيها تتغذى الفئران المصابة بداء السكري على الـ BD دون تدخل؛ المجموعات ٣-٦ (المعاملة بمستخلص الفطر GLE)، وهى الفئران المصابة بداء السكري مع الـ BD وتم التدخل بإستخدم GLE بتركيزات ٢٠٠، ٤٠٠، ٦٠٠، ٨٠٠ ملجم/ كجم من وزن الجسم عن طريق الحقن الفموي لمدة ٢٨ يومًا متتالية. كما تم الحصول على الفئران المصابة بمرض السكري من النوع ٢ عن طريق حقن الاستربتوزوتوسين (STZ). تسبب علاج الفئران بالإستربتوزوتوسين في زيادة معنوية ($p\leq0.05$) في نسبة الجلوكوز في الدم، والدهون الثلاثية (TG) ، والكوليسترول الكلي (TC) وأنواع الأكسجين التفاعلية (ROS) بنسبة ٢٠٠,١٣، ٥٥٤,٠٥، ٧١,٩٧ و٣٠,٦٢٪ مقارنة بـ المجموعة الضابطة الطبيعية على التوالي. كما تم تسجيل الاتجاه المعاكس لأنشطة كوليسترول البروتين الدهني عالي الكثافة (HDL-c) والأنزيمات المضادة للأكسدة (باروكسوناز / أريلستيراز) والتي انخفضت بنسبة – ٥٧,٩٥٪، -٥٠,٨١، -٤١,٣٢٪ مقارنة بالمجموعة الضابطة الطبيعية على التوالي. اضافة إلى ذلك، زادت تأثيرات تخثر الدم (أوقات النزف والتخثر) في فترات مختلفة من الزمن (٧، ١٤، ٢١، ٢٨ يومً). كما خفف التدخل الغذائى بمستخلص الـ GLE بشكل كبير من ارتفاع السكر وصورة دهون الدم، والـ ROS وتأثيرات تخثر الدم في الفئران المصابة بداء السكري. كما سجل معدل التحسين زيادة تعتمد على الجرعة المستخدمة من الـ GLE. وفي النهاية، توفر بيانات هذه الدراسة أساسًا لاستخدام مستخلص الفطر GLE للوقاية من أو علاج مضاعفات مرض السكري من النوع ٢ مثل ارتفاع السكر في الدم والإجهاد التأكسدي ونزيف الدم.

الكلمات المفتاحية: وزن الجسم، أنواع الأكسجين التفاعلية، الإنزيمات المضادة للأكسدة، زمن النزف، زمن التخثر.