Dietary Intervention using Reishi Mushroom (*Ganoderma Lucidum*) Reduces Oxidative Stress and Enhances Erythrocyte/Serum Defense Systems in Alloxan-Induced Diabetic Rats

Yousif A. Elhassaneen¹*, Sarah S. Aggad ² and Eman B. Meharm³

**ABSTRACT**

Diabetes mellitus (DM) is a metabolic disease that causes high blood glucose which is called hyperglycemia. In addition to hyperglycemia, oxidative stress, which raises the risk of complications, is one of the many other important factors that contribute significantly to the pathogenesis of diabetes. The goal of the current study was to determine how well (*Ganoderma lucidum*) ethanol extract (GLE) intervenes against oxidative stress in living cells, with a focus on its mechanistic elements in the management of diabetes mellitus. Rats treated with alloxan showed a significant (p≤0.01) increase in serum glucose levels from 92.67 mg/dL in normal control rats to 311.24 mg/dL in treated rats. Serum glucose levels in rats fed GLE (200, 400, 600, and 800 mg/kg bw) for 28 days showed a significant (p≤0.05) decline, with values recorded at 294.26, 268.34, 210.17, and 192.78, respectively. With GLE intervention, there was a dose-dependent increase in the rate of serum glucose decrease. The biochemical indicators of oxidative stress levels in plasma, nitric oxide (NO), malonaldehyde (MDA), and reactive oxygen species (ROS), all exhibited the same conduct. However, there was a notable (P≤0.05) increase in the various antioxidant protective mechanisms in the serum, which included bioactive molecules (GSH fractions), antioxidant vitamins (A, C, and E), and antioxidant enzymes [glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd) superoxide dismutase (SOD) and catalases (CAT)]. Ultimately, these results establish a foundation for the application of (*Ganoderma lucidum*) extracts in the prevention and/or treatment of T2D by inhibiting oxidative stress, which is one of the factors contributing to the illness and its consequent challenges.

**Keywords:** Hyperglycemia, Glutathione, Antioxidant enzymes, Antioxidant vitamins, Reactive oxygen species, Malonaldehyde, Nitric oxide.

**Graphical abstract**

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¹Department of Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt

²Department of Food and Nutrition, Faculty of Human Sciences and Design, King Abdulaziz University, Jeddah, Saudi Arabia

³Department of Home Economics, Faculty of Specific Education, Benha University, Benh, Egypt

* Corresponding Author: yousif12@hotmail.com; https://orcid.org/0000-0003-1391-3653

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease. It marks by boosted blood glucose levels that ultimately lead to crucial damage to the heart, blood vessels, kidneys, eyes, and nerves (Konstantinos et al., 2018). A chronic condition known as type 1 diabetes occurs when the pancreas yields little to no insulin on its own. The most prevalent kind, known as type 2 diabetes (T2D), strikes adults and is brought on by the body either producing too little insulin or becoming resistant to it. Approximately 537 million people worldwide suffer from diabetes, most of which reside in low- and middle-income nations (IDF, 2021). Furthermore, diabetes ranks among the world's main causes of death. Diabetes will affect five seconds of life in 2021. In accordance with anticipates from those with DM, 10.93 deaths in Egypt in 2020 or one death for every million were related to DM patients; by 2030, that number is expected to rise to 13.74 million (IDF, 2021 and WHO, 2023).

While a lot of scholarly and research institutions focus on it, the precise pathophysiology of type 2 diabetes is still unknown. As a result, researchers are trying hard to investigate the pathophysiology of type 2 diabetes, specifically how oxidative stress contributes to the development of such disease (Li & Shah, 2003; Donath & Shoelson, 2011; Dos Santos et al., 2019 and Elhassaneen et al., 2021a,b). A vital imbalance between oxidation and antioxidants, or "a disturbance in the prooxidant/antioxidant balance in favor of the former, leading to potential damage," was the original definition of oxidative stress given by Sies (1985). The most destructive free radicals and antioxidants created in living things as a result of regular cellular metabolism are known as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS) (Birben et al., 2012). Massive amounts of these oxidants, or oxidative stress, alter DNA, lipids, and proteins in cells in a detrimental manner (Halliwell & Gutteridge, 1985; Stadtman, 2004; Mahran and Elhassaneen et al., 2023). Thus, it is proposed that oxidative stress (OS) plays a role in the onset of multiple illnesses, such as diabetes (Halliwell, 1991; Ma et al., 2018 and Dos Santos et al., 2019). Human studies have shown correlations between diabetes and OS markers, such as lipid oxidative modification (Van Gaal et al., 1998). Lipid peroxidation, for instance, plays a role in the progression of atherosclerosis (Steinberg et al., 1989; Esterbauern et al., 1992 and Abd Elalal et al., 2022), a process that is known to be intensified in individuals with diabetes.

The end substances produced by the lipid peroxidation process, like malonaldehyde (MDA) and peroxides, may be detrimental to the vascular endothelium in diabetics (Pieper and Siebeneich, 1997). Furthermore, in diabetics, free radicals, such as the end products of the lipid peroxidation strike, can produce protein peroxides, which can then break down to produce other free radicals (Davies and Dean, 1997). Moreover, glycol-oxidation seems to be a major factor in the vascular endothelial dysfunction along with the nephropathy that diabetic patients expertise (Mayhan, 1997). Increased synthesis of ROS, such as O·, by endothelium may result from boosted glucose, which may mitigate the effects of nitric oxide (NO) or even form peroxynitrite, or ONOO· (Cosentino et al., 1997). On the contrary, metabolic disorders can culminate in OS, which produces ROS like superoxide anions (O2−) and hydrogen peroxide (H2O2) and disrupts insulin activity through multiple connecting pathways (Alberici et al., 2011). In the words of Evans et al. (2003), these ROS could destroy the pancreatic islets β-cells, reducing the amount of insulin released. Furthermore, ROS might stimulate a number of cellular signaling pathways, including PKC (protein kinase C) and NF-κB (nuclear factor-κB). Insulin resistance establishes as a result of ROS interfering with insulin signaling pathways (Goldin et al., 2006).

As stated by an assortment of authors, all of the detrimental physiological consequences that followed the pathogenesis of OS should be treatable with the right combination of natural and chemical antioxidants (Mayhan, 1997 and Birben et al., 2012). Although there are numerous antioxidant molecules available for the treatment of type 2 diabetes, the majority may have adverse effects that are undesirable. Furthermore, most people with limited resources and those residing in rural areas find it difficult to afford the high cost of these chemical moieties used in the management of diabetes (Ozougwu, 2011). In an attempt to address all of these issues, interest has focused on natural antioxidants, of which reishi mushrooms and other plants with medicinal properties are among the most promising sources.

The fungus Ganoderma lucidum, widely referred to as the reishi mushroom, belongs to the family Ganodermaceae of polypores that exhibit hard fruiting bodies and causes white rot and wood decay (Leskosek et al., 2010). Along with to the primary nutrients, Ganoderma lucidum’s fruiting body, mycelia, and spores contain hundreds of different bioactive compounds, primarily proteins/peptides, fatty acids, nucleotides, polysaccharides, sterols, and steroids.
Several pharmacological effects including immunomodulating, anti-atherosclerotic, analgesic, anti-inflammatory, chemopreventive, antitumor, antibacterial, antiviral, hypolipidemic, antifibrotic, liver-protective properties, preventing aging, and anti-diabetes, have been reported for *Ganoderma lucidum* due to its unique content of biologically active constituents and their biological activities (Liu et al., 1998; McKenna et al., 2002; Wasser, 2005; Aboraya et al., 2022 and Gharib et al., 2022). Moreover, beneficial for struggling diabetes-related pathologies is *Ganoderma lucidum*. *Ganoderma lucidum* ethanolic extract, for instance, has been shown by Elsemelawy et al. (2021) to boost liver and kidney functions as well as hyperglycemia in rats with type 2 diabetes. It has been suggested that *Ganoderma lucidum* extracts be used as treatments for hepatic disease due to their excellent efficacy and low toxicity (Chang & Buswell, 2008 and Sayed-Ahmed et al., 2020). Considering what we know, to our best knowledge, there have been relatively few studies that explain the mechanics and relationship between *Ganoderma lucidum* intervention and diabetes treatment. With a focus on the mechanistic aspects of *Ganoderma lucidum* ethanol extract’s (GLE) treatment of diabetes mellitus, the current study aims to determine how effective GLE is as an intervention against oxidative stress in living cells.

**MATERIAL AND METHODS**

**MATERIALS**

**Reishi mushroom**

Dried fruits of reishi mushroom (*Ganoderma lucidum*) were obtained from El-Misyria Company for Trading Herbs and Medical Plants (Haraz), Cairo, Egypt. Taxonomic confirmation of *G. lucidum* fruits was achieved by the plant taxonomy scientist in the Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt.

**Chemicals**

Sigma Chemical Co., St. Louis, MO provided the following: alloxan, γ-glutamyl glutamate, ascorbic acid, vitamin A, sulphanilamide, naphthyl ethylenediamine dihydrochloride, and thiobarbituric acid (TBA). The Morgan Company for Chemicals in Cairo, Egypt was the source of casein. El-Ghomyora Company for Trading Drugs, Chemicals, and Medical Instruments, Cairo, Egypt, provided the rest of chemicals, organic solvents and buffers. The vitamins and minerals mixtures for rat diets preparation was purchased by the same company.

**Machines**

Spectrophotometers made by Labo-med Inc. in California and Schematsu fluorescence apparatus made in Japan were used to measure the absorbance (Abs) and fluorescence (FL) for various assays. This study used a Thermo Separation Products, San Jose, CA, high-performance liquid chromatography (HPLC) system equipped with a PC 1000 system software, a UV/VIS Spectrophotometer Detector, a Consta Metvic 4100 pump, and a Spectra System FL 3000. These were the separations performed using columns (Alltech, Deerfield, IL). The reversed-phase water spherisorb ODC-2 is used for glutathione fractions. Vitamin A and E analysis was conducted using normal UltraspHERE SI and vitamin C was performed using Adsorbosil C18.

**Animals**

Animals used in the current work, adult male albino (Sprague Dawley) rats, (145 ±4.3 g per each) were purchased from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

**METHODS**

**Ganoderma lucidum ethanol extract (GLE)**

Using the methodology of Taofiq et al. (2017), the GLE was created. In a nutshell, 20 meshes per inch of dried *Ganoderma lucidum* fruits were sieved after being ground into a powder using a high-speed miller (Moulinex Egypt, Al-Araby Co., Egypt). A 5 g powder was extracted in a Soxhlet apparatus (Soxhlet Semi Automatic apparatus Velp Company, Italy) using 80% ethanol over a period of 5–6 hours (25 ± 5 min per cycle). Ethanol was evaporated in a rotary evaporator (Büchi R-210, Switzerland) to produce the dried solvent extract, which was then stored at 4 °C until required. The total yield of GLE was 1.34% (w/w) of the *Ganoderma lucidum* fruiting body.

**Biological experimental**

**Ethical approval**

The protocol of the current work was previously approved by the Scientific Research Ethics Committee, Faculty of Home Economics, Menoufia University (Approval no. 01- SREC- 03-2021).

**Basal Diet (BD)**

The following formula (per kg), as modified by AIN (1993), was used to prepare the basic diet for rats: 465.692g of corn starch, 140g of casein-85% protein, 155g of dextrinized corn starch, 100g of sucrose, 40g of soybean oil, 50g of fiber, 35g of mineral mixture, 10g of vitamin mixture, 1.8g of L-cystine, 2.5g of choline bitartrate, and 0.008g of tert-butylhydroquinone. The components of the salt mixture and vitamins that were used were prepared in accordance with AIN (1993).
**Induction of diabetes**

Normal, healthy rats were given a subcutaneous injection of freshly made alloxan monohydrate in saline at a dose of 150 mg/kg body weight to develop diabetes mellitus (Lazarow and Palay, 1954). After seven days, a drop of blood was taken from the tail vein and put through a strip of the hemoglobin test in order to measure the fast blood glucose (FBG). All of the study's animals were deemed diabetics if their FBG was greater than 200 mg/dL.

**Experimental design**

The Institute of Laboratory Animal Resources, Commission on Life Sciences of the National Research Council were followed when conducting biological experiments (NRC, 1996). Thirty-six rats were housed individually in wire cages in a room that was kept at a constant temperature of 24 ± 2.8 °C. Prior to the start of the experiment, all rats were given a BD for one week in order to acclimate them. The rats were then split into two main groups: 30 rats were used for the diabetes induction and were further divided into five subgroups as follows: the first group, which was the normal control group (Group 1, 6 rats) and was still fed on BD, group (2), model control, fed on BD only as a positive control (rats with diabetes) and groups (3-6) fed on BD beside administered by oral gavages, using a feeding needle with 200, 400, 600, and 800 mg/kg bw Ganoderma lucidum ethanol extract (GLE), respectively. GLE doses used here based on based our previous studies (Elhassaneen et al., 2016b; Sayed-Ahmed et al., 2020 and Elsemelawy et al., 2021). For 28 days, each of the above groups was housed in a single cage. Rats were weighed at the start of the trial, then weekly, and finally after the experiment. The body weight gain (BWG, %), food intake (FI), and food efficiency ratio (FER) were determined according to Chapman et al. (1959).

**Blood sampling**

After a 12-hour fast, blood samples were taken using the abdominal aorta at the end of the 28-day experiment, and rats were sacrificed while sedated with ether. Glass centrifuge tubes were used to receive the blood samples. Following a 10-minute centrifugation at 3000 rpm, plasma was diluted and utilized to analyze antioxidant vitamins A, C, and E. After three consecutive washes with a 0.9% NaCl solution, the erythrocyte residue was haemolyzed for 30 minutes using deionized water. After centrifuging the haemolysate for 30 minutes at 20000 xg, the supernatant fractions were moved to a clean test tube and the antioxidant enzymes GSH-Px, GSH-Rd, SOD, and CAT were examined (Stroev and Makarova, 1989).

**Hematological analysis**

**Serum glucose**

Serum glucose was determined by the colorimetric method explained by Tietz (1976).

**Serum lipid profile**

Triglycerides (TGs), Total cholesterol (Cho), high density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined in serum according to the standard methods described by Fossati & Prenapa (1982); Richmod (1973); Lopes-Virella et al. (1977) and Schriewer et al. (1984), respectively.

**Glutathione fractions**

Samples for reduced (GSH) and oxidized (GSSG) glutathione determination were prepared, extracted, and injected into the HPLC system according to the method of McFarris and Reed (1987).

**Antioxidant enzymes**

Glutathione peroxidase (GSH-Px) and catalase (CAT) activities were determined as mentioned by Splittgerber & Tappel (1979) and Aebi (1974), respectively. Superoxide dismutase (SOD) activity was determined by a colorimetric assay kit (Creative BioLab, NY) according to the method of Mett and Müller (2021). The International Committee for Standardization in Haematology recommended a method for determining GSH-Rd activity (ICSH, 1979).

**Antioxidant vitamins**

All vitamins A, C (ascorbic acid), and E (α-tocopherol) were extracted, purified, elution, and analyzed by HPLC techniques by adaptation of the methods of Epler et al. (1993); Moeslinger et al. (1994) and Hung et al. (1980), respectively.

**Biological oxidant parameters**

Reactive oxygen species (ROS) was determined by a colorimetric method described by Erel (2005). Malonaldehyde (MDA) content was measured as described by Buege and Aust (1978). Nitric oxide (NO) was estimated as the total of NO2 and NO3 according to Miranda et al. (2001).

**Statistical Analysis**

Each statistic was done three times, and the results were reported as mean +/- standard deviation (SD). The Student t-test and MINITAB-12 software (Minitab Inc., State College, PA, USA) were used for statistical analysis.
RESULTS AND DISCUSSION

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

Table (1) and Figure (1) demonstrate the impact of *Ganoderma lucidum* ethanol extract (GLE) intervention on BWG, FI, and FER of GLE. According to these data, BWG, FI, and FER were detected by normal control rats at 0.917%, 12.20 g/day/rat, and 0.081, respectively. With respect to the normal group, the rats treated with alloxan showed significantly (p≤0.05) lower BWG (-40.04), FI (-31.01), and FER (-18.73). When feeding rats GLE (200, 400, 600, or 800 mg/kg bw) for 28 days, the levels of BWG, FI, and FER significantly (p≤0.05) increase. With the GLE enforcement, there was a dose-dependent increase in the rate of increase in BWG, FI, and FER. Whether the data are from *G. lucidum* or other genera of algae, the current data are almost identical with those reported by multiple authors (Elhssaneen et al., 2019 & 2020; El-Gamal, 2020; Sayed-Ahmed et al., 2020 and Elsemelawy et al., 2021). In relation to these studies, the high concentration of bioactive compounds (phenolics, polysaccharides, flavonoids, dietary fiber, and lycopene) in GLE and their associated biological activities (antioxidant and scavenging activities) are what prompted the decrease in BWG, FI, and FER following the intervention. Furthermore, a variety of studies have revealed that BW, FI, and FER considerably reduce in liver/kidney rat disorders that are probably triggered by diabetes (Hamzawy et al., 2013; Abd El-Rahman 2021; Elsemelawy et al., 2021 and Elhassaneen et al., 2024). Utilizing plant parts containing bioactive compounds, like those in GLE, has been displayed to improve these disorders. Furthermore, it was demonstrated by Morresion and Hark (1999) that malnutrition, which consists of insufficient food intake, improper digestion, malabsorption, and anomalies in the metabolism and storing of nutrients, can result from diabetes and liver diseases.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>BWG (%)</th>
<th>FI (g/day/rat)</th>
<th>FER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.917± 0.112a</td>
<td>12.20± 0.94a</td>
<td>0.081± 0.007a</td>
</tr>
<tr>
<td>Model control</td>
<td>0.550± 0.131d</td>
<td>8.42± 1.04c</td>
<td>0.066± 0.021c</td>
</tr>
<tr>
<td>GLE intervention (200 mg/kg BW)</td>
<td>0.607± 0.091c</td>
<td>9.12± 0.90c</td>
<td>0.068± 0.019bc</td>
</tr>
<tr>
<td>GLE intervention (400 mg/kg BW)</td>
<td>0.689± 0.081c</td>
<td>9.40± 0.83c</td>
<td>0.072± 0.014b</td>
</tr>
<tr>
<td>GLE intervention (600 mg/kg BW)</td>
<td>0.731± 0.067bc</td>
<td>9.78± 0.72c</td>
<td>0.073± 0.012b</td>
</tr>
<tr>
<td>GLE intervention (800 mg/kg BW)</td>
<td>0.790± 0.103b</td>
<td>10.71± 0.95b</td>
<td>0.076± 0.017b</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n=6). Means on the same column with superscript letters indicate a significant difference at P ≤ 0.05. Normal control: healthy rats without intervention; Model control: alloxan-induced diabetic rats without intervention; GLE intervention: alloxan-induced diabetic rats with GLE intervention. BWG: body weight gain; FI: feed intake (FI); FER: feed efficiency ratio.
The effect of GLE intervention on serum glucose of diabetic rats

Table (2) displays the impact of GLE intervention on the alloxan-induced serum glucose level in diabetic rats. According to these data, rats treated with alloxan recorded 311.24 mg/dL, a significant (p≤0.01) increase from the 92.67 mg/dL recorded by typical control rats. After feeding rats GLE (200, 400, 600, and 800 mg/kg bw) for 28 days, the normal control group's serum glucose levels decreased at a significant (p≤0.05) rate to 235.86, 217.54, 217.54, 189.57, 126.79, and 108.03, respectively.

The diabetic rats' serum glucose levels decreased at a rate that was dose-dependent in response to the GLE intervention. Alloxan can induce chronic or enduring diabetes in experimental animals by selectively destroying pancreatic islet β-cells (Mathe, 1995 and Elsemelawy et al., 2021). Certain kinds of reactive oxygen species (ROS) are produced by it, which assault DNA and cause β-cell DNA strand breaks, leading to the β-cell’s eventual demise (Puszta & et al., 1996 and Lenzen, 2008). By decreasing the uptake of glucose by peripheral tissues and glycogenolysis while increasing gluconeogenesis and hepatic glucose production, this will result in hyperglycemia (Caro, 1990; Beck-Nielsen, 2002 and Jung et al., 2011). This theory aligns with the findings of Elsemelawy et al. (2021), who noticed that rats treated with alloxan had substantially higher serum glucose levels and significantly lower serum insulin levels. Consequently, the hypoglycemic effect of GLE in alloxan-induced diabetic rats observed in this study may be caused by the various biological activities of GLE as a result of its plenty of biologically active substances. Phenolics, lycopene, polysaccharides, terpenoids, flavonoids, triterpenoids, cyclo-octasulfur, ergosterol peroxide, cerebrosides, sterols, and vitamins A, B, and E have all been found to be high in G. lucidum (Mizuno, 1995; McKenna et al., 2002; Gao et al., 2004 and Liu et al., 2016). According to Elhassaneen et al. (2012 & 2015); Elmaadawy et al. (2016); Ali et al. (2017); Elbasoumy et al. (2019) and Elhassaneen et al. (2023), these chemical compounds are recognized for their potent biological effects, which include antioxidant activities, inhibition of lipid oxidation and ROS scavenging activity, improved glucose response, and relief of metabolic dysregulation of free fatty acids and insulin resistance linked to type 2 diabetes. Furthermore, Tiwari and Rao (2002) reviewed the bioactive compounds found in G. lucidum, such as polyphenolics, which have been shown to inhibit α-amylase and sucrase in addition to their well-known antioxidant qualities. These compounds are the main agents that reduce postprandial blood sugar levels.

Additionally, Wasser (2005) has shown that in experimental animals, the polysaccharide fractions of Ganoderma lucidum may have hypoglycemic and hypolipidemic effects. This suggested mechanism is consistent with the research findings of Elsemelawy et al. (2021), which showed that in diabetic rats undergoing GLE intervention, serum insulin levels significantly increased while serum glucose levels significantly decreased when contrasted with alloxan-diabetic rats.

Effect of GLE intervention on serum lipid profile of diabetic rats

Table (3) and Figures (2) illustrate its effect of GLE on the serum lipid profiles of diabetic rats. Based on this information, it was possible to determine that rats treated with alloxan had serum triglycerides (TGs), total cholesterol (TC), and low density lipoprotein cholesterol (LDL-c) significantly higher (p≤0.05) in comparison to normal control animals by the ratios of 34.93, 64.36, and 47.64%, respectively. The level of high-density lipoprotein cholesterol (HDL-c) showed a significant (p≤0.05) decrease of -39.27%, in the opposite direction. GLE intervention at 200, 400, 600, and 800 mg/kg bw/day in rat diets for 28 days resulted in a significant (p≤0.05) reduction in TG, TC, and LDL-c levels at varying rates in comparison to the normal control group. With regard to HDL-c, the opposite trend was noted. As a result of the GLE intervention, the diabetic rats’ serum TG, TC, and LDL-c levels decreased at a rate that increased in HDL-c in dose-dependent ways. The current study's data thus indicated that diabetes mellitus (DM) was linked to hyperlipidemic (TGs) and hypercholesterolemic (TC) conditions, meaning that TGs, TC, and LLGL-c increased serum bad lipid particles and HDL-c decreased serum good lipid parts. Significantly (p≤0.05) improved serum good lipid elements following GLE intervention. Authors (Elhassaneen et al., 2021 a;c; Shalaby & Elhassaneen, 2021; Elhassaneen et al., 2022 a,b; Aboraya et al., 2022 and Gharib et al., 2022) observed the same conduct with different pant regions than Ganoderma lucidum. In light of this, Badawy (2008) reviewed how high TG and LDL-c levels increase the risk of atherosclerosis, peripheral vascular disease, fatty liver, and cardiovascular disease in people. During a period of time, large chemical-based oral antihyperlipidemic and antihypercholesterolemic pharmaceuticals were created; however, virtually all of them have unfavorable side effects.
Table 2. Effect of GLE intervention on serum glucose level of diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum glucose conc.</th>
<th>Percent of normal control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>92.67±5.12abc</td>
<td>------</td>
</tr>
<tr>
<td>Model control</td>
<td>311.24± 17.46a</td>
<td>235.86</td>
</tr>
<tr>
<td>GLE intervention (200 mg/kg b w)</td>
<td>294.26± 8.56b</td>
<td>217.54</td>
</tr>
<tr>
<td>GLE intervention (400 mg/kg b w)</td>
<td>268.34±10.54c</td>
<td>189.57</td>
</tr>
<tr>
<td>GLE intervention (600 mg/kg b w)</td>
<td>210.17±10.37d</td>
<td>126.79</td>
</tr>
<tr>
<td>GLE intervention (800 mg/kg b w)</td>
<td>192.78±14.71d</td>
<td>108.03</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n=6 rats). Means on the same column with superscript letters indicate a significant difference at P ≤ 0.05. Data for the experimental groups are as under Table 1.

Table 3. Effect of GLE intervention on serum lipid profile of diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>TGs (mg/dL)</th>
<th>Cho (mg/dL)</th>
<th>LDL-c (mg/dL)</th>
<th>HDL-c (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>127.21±2.90d</td>
<td>134.65±3.89e</td>
<td>129.98±6.18d</td>
<td>51.87±4.19a</td>
</tr>
<tr>
<td>Model control</td>
<td>171.65±6.01a</td>
<td>221.31±6.21a</td>
<td>191.90±5.19a</td>
<td>31.50±5.76c</td>
</tr>
<tr>
<td>GLE intervention (200 mg/kg BW)</td>
<td>165.01±4.65a</td>
<td>210.17±3.90a</td>
<td>187.88±4.82ab</td>
<td>31.98±3.91c</td>
</tr>
<tr>
<td>GLE intervention (400 mg/kg BW)</td>
<td>157.16±6.98ab</td>
<td>197.33±4.65b</td>
<td>179.65±3.98b</td>
<td>37.45±6.04b</td>
</tr>
<tr>
<td>GLE intervention (600 mg/kg BW)</td>
<td>148.21±4.17c</td>
<td>179.90±4.87c</td>
<td>164.56±4.70b</td>
<td>44.04±3.71ab</td>
</tr>
<tr>
<td>GLE intervention (800 mg/kg BW)</td>
<td>141.17±5.12cd</td>
<td>156.73±6.31d</td>
<td>154.70±6.42c</td>
<td>48.32±2.97a</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n=6). Means on the same column with superscript letters indicate a significant difference at P ≤ 0.05. Data for the experimental groups are as under Table 1. TG: Triglycerides; Cho: Cholesterol; HDL: High-density lipoproteins cholesterol; LDL: Low-density lipoproteins cholesterol.

The findings of this study proposed that, without any discovered adverse effects, *Ganoderma lucidum* might be a useful natural substitute for managing diabetes mellitus that improves serum lipid profiles. *Ganoderma lucidum* extracts were found to have an anti-atherosclerotic effect by Gharib et al. (2022) based on those data. Additionally, *Ganoderma lucidum* revealed antihyperlipidemic and antihypercholesterolemic effects via one or more of the following routes: it minimized the absorption of cholesterol and the amount of VLDL and TGs in the liver. Reducing the VLDL level as a result of *Ganoderma lucidum* interference was linked to a drop in VLDL establishment and release from the liver as well as an inhibition of intestinal cholesterol absorption, according to the identical research with other (Aboraya et al., 2022). A lower risk of heart disease and many chronic aging diseases was also linked to diets high in plant foods (fruits and vegetables) as reported by a number of authors (Bedawy, 2008; Elhassaneen et al., 2020 and Shalaby & Elhassaneen, 2021). According to Abd Elalal (2022) their research in *Ganoderma lucidum*, these foods consist of botanical compounds. The results of this study, along with others, suggested that *Ganoderma lucidum*’s potential...
mechanism of action to enhance blood lipid profile could be explained by one or more of the following mechanisms: increase antioxidant and anti-inflammatory activities (Elbasouny et al., 2019; Almutairiu, 2020 and Sayed, 2020); reduce LDL oxidation and endothelial cell damage, which is thought to be involved in the initial phases of atherosclerosis (Aviram & Vaya, 2013 and Bedawy, 2008); hinder intake of antioxidant vitamins like α-tocopherol, protect human serum paraxonase (PON 1) tasks (Aviram & Vaya, 2013 and Aboraya et al., 2022); bind to albumin and never integrated into the LDL particle and mitigate intestinal cholesterol absorption through polymeric structure of some *Ganoderma lucidum* bioactive compounds such as polyphenols and polysaccharides that could attach to cholesterol and bile acids (Wasser, 2005; Sayed-Ahmed et al., 2020; Elsemelawy et al., 2021 and Gharib et al., 2022). As consequently, *Ganoderma lucidum*’s hypolipidemic and hypocholesterolemic features make it an intriguing therapy for CVDs like atherosclerosis.

### The effect of GLE intervention on antioxidative defense systems of diabetic rats

#### Glutathione fraction levels in plasma

In diabetic rats induced by alloxan, the impact of GLE intervention on glutathione fractions, biological antioxidant macromolecules, was evaluated as shown in Table 4 and Figure 3. According to the evidence, the GSH and GSSG levels of normal control rats were 8.04 and 0.654 µmol/L, respectively. GSH (-26.89%) and GSSG (-9.48%) were considerably lower in the alloxan-treated rats than in the control group (p≤0.05). The levels of GSH and GSSG substantially (p≤0.05) increase after feeding rats GLE (200, 400, 600, and 800 mg/kg bw) for 28 days. The diabetic rats' rate of increase in GSH and GSSG following GLE intervention was shown to be dose-dependent. L-glutamyl-L-cysteinyl-glycine, or GSH, is a tripeptide that is found in mill molar quantities in every cell and is a significant antioxidant (Lu, 1999). Among its many antioxidant features is its involvement in the activities of the GSH enzyme family, which includes peroxiredoxins (PRXs), glutathione reductase (GSH-Rd), and glutathione peroxidase (GSH-Px). Furthermore, GSH has the ability to neutralize oxyradicals without the need for enzymes (Halliwell and Gutteridge, 1985). Moreover, GSH depletion has been linked to a number of oxidative injuries (Musallam et al., 2002). The associations between reduced levels of GSH and hyperglycemia has been stated in a number of studies (Aly et al., 2017 and Elhassaneen et al., 2021a, b). According to Konstantinos et al. (2018), the interaction can be clarified as follows: under hyperglycemia, glucose is particularly used in the polyol pathway, which consumes NADPH required for GSH recuperation by the GSH-Rd enzyme. Thus, the loss of GSH is caused inadvertent by hyperglycemia.

#### Table 4. Effect of GLE intervention on glutathione fractions of diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (µmol/L)</th>
<th>GSSG (µmol/L)</th>
<th>GSH/GSSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>8.04 ± 0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.654 ± 0.060&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.29 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Model control</td>
<td>5.88 ± 0.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.592 ± 0.076&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.93 ± 0.78&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GLE intervention (200 mg/kg BW)</td>
<td>6.45 ± 1.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.602 ± 0.030&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.71 ± 0.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GLE intervention (400 mg/kg BW)</td>
<td>6.59 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.603 ± 0.062&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.93 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GLE intervention (600 mg/kg BW)</td>
<td>7.11 ± 1.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.604 ± 0.085&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.77 ± 0.40&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>GLE intervention (800 mg/kg BW)</td>
<td>7.39 ± 1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.625 ± 0.102&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.82 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n=6 rats). Means on the same column with superscript letters indicate a significant difference at P≤0.05. Data for the experimental groups are as under Table 1. GSH; Reduced glutathione; GSSG: oxidized glutathione.
Conversely, the same data also showed that the drop in glutathione fractions (GSH and GSSG) observed in rats with diabetes treated with alloxan was generally accompanied by a corresponding reduction in the GSH/GSSG ratio. Rats with diabetes treated with alloxan had a GSH/GSSG ratio of 9.93, a significant decrease from 12.29 in normal rats. GLE (200, 400, 600, and 800 mg/kg bw) is fed to rats for 28 days, and the ratio of GSH/GSSG increases significantly (p≤0.05). According to a number of studies (Elhassaneen & Abd El-Moaty, 2003; Elhassaneen, 2004 and Aly et al., 2017), increased fluxes of oxy-radicals may be decreased in the GSH/GSSG ratio due to either direct detoxification of radicals or increased peroxidase activity. Reduced NADPH availability, which is required for GSH-Rd activity, as a result of oxidations in the first step of the reactive oxygen species cycle may also have an indirect effect (Champe and Harvey, 1994). One of the main causes of the GSH/GSSG ratio decline in diabetic rats may be assigned to the generated ROS by a variety of cellular enzymes, including NAPDH oxidases (NOX) (Bedard and Krause, 2007).

Antioxidant enzyme activities in red blood cells (RBCs)

Table (5) and Figure (4) reveal the effect of GLE intervention on the antioxidant enzyme activities of erythrocytes in diabetic rats stimulated by alloxan. The analysis showed that the glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), catalase (CAT), and superoxide dismutase (SOD) measurements for normal control rats were 26.32, 11.14, 171.75, and 6.11 U/g Hb, respectively. GSH-Px (-36.25%), GSH-Rd (-26.93%), CAT (-22.83%), and SOD (-19.80%) were all significantly (p≤0.05) lower in the alloxan-treated rats than in the control group. Increases in GSH-Px, GSH-Rd, CAT, and SOD levels are significant (p≤0.05) when feeding rats GLE (200, 400, 600, and 800 mg/kg bw) for 28 days. GLE intervention increased the rate of increase in antioxidant enzymes (GSH-Px, GSH-Rd, CAT, and SOD) in depending on the dose. Antioxidant defense systems, mostly centered on antioxidant enzymes (GSH-Px, GSH-Rd, CAT, and SOD) that can eliminate ROS and avoid free radical damages (OS activities), are known to have been developed by a living thing. O2− is converted by SODs to H2O2, which follows elimination by an array of enzymes, including CAT and GSH-Px (McCord et al., 1976). Furthermore, the reduced form of the enzyme interacts with H2O2 after the GSH reduces the Se. In normal cells, the nine GHS/GSSG ratio remains high. Thus, a method for lowering GSSG back to GSH has to exist. The GSH-Rd enzyme facilitates the following reaction: GSSG + NADPH + H+ → 2GSH + NADP+. In order to produce NADPH for GSH reduction, mammalian cells RBCs operate the pentose phosphate pathway. Furthermore, GSH-Rd is capable of stimulating the reduction of mixed disulfides, such as those involving GSH and Co-Enzyme A (Lu, 1999). Multiple studies have demonstrated the function of antioxidant enzyme systems in hepatic cells (Elhassaneen, 1996; Galinier et al., 2004 and Cao, 2014). Reduced antioxidant enzyme activity triggers more ROS production and dysfunction in mitochondria in both in vitro and in vivo systems (Elhassaneen, 1996 and Curtis et al., 2010). Wealthy in bioactive compounds like phenolics, carotenoids, polysaccharides, flavonoids, etc., the chosen GLE for the current study strategies has demonstrated antioxidant activity in various kinds of biological systems (Wachtel-Galor et al., 2011 and Sayed Ahmed et al., 2020). Through ROS scavenging mechanisms in red blood cells, these antioxidant activities play a significant role in regulating the progression and adverse effects of diabetes.

Table 5. Effect of GLE intervention on erythrocytes antioxidant enzyme activities of diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH-Px (U/g Hb)</th>
<th>GSH-Rd (U/g Hb)</th>
<th>CAT (U/g Hb)</th>
<th>SOD (U/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>26.32 ± 2.20</td>
<td>11.14 ± 1.74</td>
<td>171.75 ± 10.56</td>
<td>6.11 ± 1.71</td>
</tr>
<tr>
<td>Model control</td>
<td>16.78 ± 2.35</td>
<td>8.14 ± 0.93</td>
<td>132.54 ± 12.17</td>
<td>4.90 ± 0.99</td>
</tr>
<tr>
<td>GLE intervention (200 mg/kg BW)</td>
<td>17.98 ±2.31 ∧</td>
<td>8.39 ± 1.54</td>
<td>139.54 ± 8.67</td>
<td>4.97 ± 1.30</td>
</tr>
<tr>
<td>GLE intervention (400 mg/kg BW)</td>
<td>20.76 ±3.01 b</td>
<td>8.47 ± 1.72</td>
<td>147.73 ± 14.86</td>
<td>4.99 ± 1.69</td>
</tr>
<tr>
<td>GLE intervention (600 mg/kg BW)</td>
<td>21.54 ±3.66 c</td>
<td>9.65 ± 1.21</td>
<td>151.84 ± 5.99</td>
<td>5.32 ±1.19</td>
</tr>
<tr>
<td>GLE intervention (800 mg/kg BW)</td>
<td>22.98 ±1.70 b</td>
<td>10.61 ± 2.21</td>
<td>157.54 ± 16.62</td>
<td>5.79 ± 1.89</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n=6 rats). Means on the same column with superscript letters indicate a significant difference at P ≤ 0.05. Data for the experimental groups are as under Table 1. GSH-Px: glutathione peroxidase; GSH-Rd: glutathione reductase; CAT: catalase; SOD: superoxide dismutase.
Antioxidant vitamins concentration in plasma

Table (6) and Figure (5) show the impact of GLE intervention on the plasma antioxidant vitamins of diabetic rats induced by alloxan. According to these data, the levels of vitamins A, C, and E in normal control rats were 1.43, 62.11, and 32.67 µmol/L, respectively. In contrast to the alloxan-treated rats to the control group, there was a significant (p≤0.05) drop in vitamin A (-8.39%), vitamin C (-21.28%), and vitamin E (-23.45%). Vitamin A, C, and E levels in feeding rats increased significantly (p≤0.05) after 28 days of GLE (200, 400, 600, and 800 mg/kg bw) intervention. With the GLE intervention, the rate of increase in antioxidant vitamins (A, C, and E) displayed an influence by dose increase. Furthermore, to red blood cells, plasma is plentiful in naturally occurring antioxidant substances like β-carotenoids and lipid-soluble vitamins (like A and E) (Wohaib and Godin, 1987). Indirect reports of vitamin A’s possible antioxidant function suggest that it may help guard the body from OS damage. Retinoic acid, a metabolite of vitamin A, has been shown to increase the activity of glutathione transferase and SOD while lowering MDA and ROS in both treated and untreated models. These findings suggest that retinol enhances the activity of antioxidant enzymes (Malivindi et al., 2018). Effective antioxidants like vitamin C may neutralize free radicals both inside and outside of cells by either directly combating peroxyl radicals or indirectly doing so by enhancing vitamin E’s antioxidant capacity. This mechanism aids in the regulation of lipid peroxidation of nuclear materials and cellular membranes. Because of its well-known antioxidant qualities, ascorbate reduces ROS or defends proteins that aid in DNA repair, thus minimizing the damage to DNA (Padayatty et al., 2003). Olorunnisola et al. (2019) also reviewed the evidence supporting vitamin C’s ability to ameliorate OS and diabetes complications. Vitamin E is a substance soluble in fat. It was discovered that in exercise-induced OS, an elevated level of vitamin E in the liver provided protection against oxidative damage (Górnicka et al., 2016). Additionally, by scavenging lipid peroxyl free radicals and superoxide radical anion, vitamin E shields cell membranes from lipid peroxidation (Prokopowicz et al., 2013). In diabetic wounds, vitamin E has also been demonstrated to reduce inflammation, OS, and apoptosis (Shin Jihyun et al., 2017). As per the findings of Wachtel-Galor et al. (2011) and Sayed Ahmed et al. (2020), the GLE that has been picked for the current study intervention is abundant in bioactive compounds, such as carotenoids and vitamins, which have demonstrated antioxidant activities in different ecosystems. Since ROS scavenging processes increase the bioavailability of vitamins in plasma cells, such antioxidant properties are crucial in manipulating the development of diabetes.

Effect of GLE intervention on serum reactive oxygen species (ROS) levels of diabetic rats

By measuring the amount of reactive oxygen species (ROS) in the serum, the oxidative stress status in diabetic rats treated with GLE was evaluated (Table 7). These data showed that, in comparison to the normal control animal, alloxan-induced a significant increase (p≤0.05) in ROS concentration in serum, 34.07%. In comparison to the normal control animals, the rates of ROS were significantly (p≤0.05) decreased after 28 days of GLE (200, 400, 600, and 800 mg/kg bw/day) intervention in the rat feeding protocol. The reductions were 31.60, 27.15, 16.90, and 10.74%, respectively. The diabetic rats' serum ROS decreased at a rate that was dose-dependent following the GLE intervention. A common biological marker for oxidative stress status that offers an accurate indicator is ROS (Aboraya et al., 2020).
Yousif A. Elhassaneen et al. (2022; Elhassaneen et al., 2023). The current study’s data verified that the GLE intervention’s reduction of ROS raised the reactive oxygen species level in diabetic rats. According to earlier research, there is a direct correlation between serum ROS concentration and various disease pathological stages, including DM (Elmaadawy et al., 2016; Elsemelawy et al., 2021 and Elhassaneen et al., 2022 a,b). The increase of oxidative stress is additionally promoted by systemic metabolic changes linked to DM, including hyperglycemia, liver and kidney abnormalities, and altered serum lipid profiles in experimental animals (Elsemelawy et al., 2021; Elhassaneen et al., 2016a & 2021c and 2022a). Consequently, regulating the level of ROS in serum as a consequence of GLE intervention would be useful in preserving an appropriate degree of oxidative stress. The results of this study, along with others, suggested that *Ganoderma lucidum*’s potential mechanism of ROS reduction may be linked to the antioxidant and free radical scavenging features of its compounds that are bioactive (Wasser, 2005 and Gharib et al., 2022). Additionally, superoxide dismutase, glutathione peroxidase, catalase, and other antioxidant enzyme activity may be enhanced by *Ganoderma lucidum*, which also prevents lipid peroxidation (Wasser, 2005; Elsemelawy et al., 2021 and Gharib et al., 2022).

**Table 6. Effect of GLE intervention on serum antioxidant vitamins of diabetic rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Vitamin A (Retinol, μmol/L)</th>
<th>Vitamin C (Ascorbic acid, μmol/L)</th>
<th>Vitamin E (Tocopherol, μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.43 ± 0.16 a</td>
<td>62.11 ± 3.67 a</td>
<td>32.67 ± 3.65 a</td>
</tr>
<tr>
<td>Model control</td>
<td>1.31 ± 0.18 b</td>
<td>48.89 ± 9.15 c</td>
<td>25.01 ± 5.10 c</td>
</tr>
<tr>
<td>GLE intervention (200 mg/kg BW)</td>
<td>1.33 ± 0.14 a</td>
<td>53.03 ± 6.02 b</td>
<td>27.01 ± 2.56 b</td>
</tr>
<tr>
<td>GLE intervention (400 mg/kg BW)</td>
<td>1.37 ± 0.24 a</td>
<td>54.90 ± 5.67 b</td>
<td>27.15 ± 3.95 b</td>
</tr>
<tr>
<td>GLE intervention (600 mg/kg BW)</td>
<td>1.38 ± 0.22 a</td>
<td>58.56 ± 8.05 ab</td>
<td>29.12 ± 1.98 ab</td>
</tr>
<tr>
<td>GLE intervention (800 mg/kg BW)</td>
<td>1.39 ± 0.31 a</td>
<td>59.90 ± 6.11 a</td>
<td>30.11 ± 3.02 a</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n=6 rats). Means on the same column with superscript letters indicate a significant difference at P ≤ 0.05. Data for the experimental groups are as under Table 1.

**Table 7. Effect of GLE intervention on serum reactive oxygen species (ROS) concentration of diabetic rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Value (U/mL)</th>
<th>Percent of change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>59.34± 4.98 a</td>
<td>-----</td>
</tr>
<tr>
<td>Model control</td>
<td>79.56± 7.67 a</td>
<td>34.07</td>
</tr>
<tr>
<td>GLE intervention (200 mg/kg b w)</td>
<td>78.09± 7.45 a</td>
<td>31.60</td>
</tr>
<tr>
<td>GLE intervention (400 mg/kg b w)</td>
<td>75.45± 5.98 ab</td>
<td>27.15</td>
</tr>
<tr>
<td>GLE intervention (600 mg/kg b w)</td>
<td>69.37± 8.11 b</td>
<td>16.90</td>
</tr>
<tr>
<td>GLE intervention (800 mg/kg b w)</td>
<td>65.71± 5.55 bc</td>
<td>10.74</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n = 6). Different superscript letters on the same column indicate significant difference (P ≤ 0.05). Data for the experimental groups are as under Table 1.
Effect of GLE intervention on serum oxidants of diabetic rats

In diabetic rats given alloxan, the impact of GLE interference on the concentration of plasma oxidants (i.e., oxidative stress) was evaluated in Table (8) and Figure (6). These results showed that the MDA and NO levels in normal control rats were 5.96 nmol/L and 48.56 µM/L, respectively. With regard to the normal group, the rats treated with alloxan showed a significant (p≤0.05) increase in MDA (84.73%) and NO (159.35%). Rats treated with GLE (200, 400, 600, and 800 mg/kg bw) for 28 days show a significant (p≤0.05) decreased MDA and NO levels. With the GLE intervention, the rate of decline of oxidants (MDA and NO) showed an increase that was dose-dependent.

Evaluations of biological indicators or end products of free radical-mediated oxidative processes have been used in similar studies to provide clinical evidence for diabetes-associated OS (Elhassaneen, 2004; Elhassaneen et al., 2014, 2021a and Sayed Ahmed, 2016). Lipid peroxidation markers, for example, attached dienes, lipid hydro peroxides, and malondialdehyde (MDA), which are major products of the oxidation of polyunsaturated fatty acids, are found to be elevated in the plasma of subjects with diabetes in survey clinical studies (Elhassaneen and Salem 2014). Concern over MDA’s possible effects on human health has been obtained by reports that it is a mutagenic and carcinogenic chemical (Shamberger et al., 1974). Conversely, NO is a tiny molecule that is crucial for liver cell-to-liver communication and for the regulation of key liver activities (Manahan, 1989). Through the utilization of nitric oxide synthase to catalyze the conversion of L-arginine to citrulline and the incredibly reactive free radical species nitric oxide (NO), it is produced. According to Manahan (1989) and Misko et al. (1993), NO can also react with hemoglobin to form iron-nitrosyl adducts and/or nitrate in blood, with superoxide anion to make nitrite, and with amino and thiol groups of protein to form nitrosylated species. A growing variety of inflammatory and immunological disorders, including diabetes, have been linked to increased nitric oxide production in their disease progression and tissue damage (Jacob et al., 1992; Elhassaneen et al., 2014; Aly et al., 2017 and Yousuf et al., 2019). Consistent with previous research, the GLE chosen for the current study intervention is abundant in bioactive substances, such as vitamins, carotenoids, flavonoids, and phenolics, all of which have demonstrated antioxidant properties in various biological systems (Wachtel-Galor et al., 2011 and Sayed Ahmed et al., 2020). Via ROS scavenging mechanisms and by preventing lipid oxidation in plasma, these antioxidant qualities play a significant role in the management of diabetes and the emergence of its complications.

Table 8. Effect of GLE intervention on serum oxidants of diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nM/mL)</th>
<th>NO (µM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5.96 ± 0.810 c</td>
<td>48.56 ± 2.19 e</td>
</tr>
<tr>
<td>Model control</td>
<td>11.01 ± 1.110 a</td>
<td>125.94 ± 7.71 a</td>
</tr>
<tr>
<td>GLE intervention (200 mg/kg BW)</td>
<td>9.62 ± 0.620 ab</td>
<td>119.02 ± 7.64 b</td>
</tr>
<tr>
<td>GLE intervention (400 mg/kg BW)</td>
<td>8.76 ± 0.810 b</td>
<td>102.56 ± 5.81 c</td>
</tr>
<tr>
<td>GLE intervention (600 mg/kg BW)</td>
<td>8.13 ± 0.490 b</td>
<td>99.78 ± 9.62 c</td>
</tr>
<tr>
<td>GLE intervention (800 mg/kg BW)</td>
<td>7.86 ± 0.860 bc</td>
<td>91.45 ± 6.65 d</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n = 6 rats). Means on the same column with superscript letters indicate a significant difference at P ≤ 0.05. Data for the experimental groups are as under Table 1. MDA: malondialdehyde; NO: nitric oxide.

Fig. 6. Effect of GLE intervention on serum oxidants of diabetic rats
Table 9. Correlation between oxidants and antioxidant defense systems parameters in diabetes rat’s intervention with GLE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$r^2$</th>
<th>Parameters</th>
<th>$r^2$</th>
<th>Parameters</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA/GSH</td>
<td>-0.9381</td>
<td>NO/GSH</td>
<td>-0.9143</td>
<td>ROS/GSH</td>
<td>-0.9437</td>
</tr>
<tr>
<td>MDA/GSSG</td>
<td>-0.9136</td>
<td>NO/GSSG</td>
<td>-0.8680</td>
<td>ROS/GSSG</td>
<td>-0.8942</td>
</tr>
<tr>
<td>MDA/Vit A</td>
<td>-0.8690</td>
<td>NO/Vit A</td>
<td>-0.8439</td>
<td>ROS/Vit A</td>
<td>-0.8715</td>
</tr>
<tr>
<td>MDA/Vit C</td>
<td>-0.8151</td>
<td>NO/Vit C</td>
<td>-0.7656</td>
<td>ROS/Vit C</td>
<td>-0.8371</td>
</tr>
<tr>
<td>MDA/Vit E</td>
<td>-0.8943</td>
<td>NO/Vit E</td>
<td>-0.8672</td>
<td>ROS/Vit E</td>
<td>-0.8712</td>
</tr>
<tr>
<td>MDA/GSH-Px</td>
<td>-0.9476</td>
<td>NO/GSH-Px</td>
<td>-0.9230</td>
<td>ROS/GSH-Px</td>
<td>-0.9520</td>
</tr>
<tr>
<td>MDA/GSH-Rd</td>
<td>-0.9129</td>
<td>NO/GSH-Rd</td>
<td>-0.8917</td>
<td>ROS/GSH-Rd</td>
<td>-0.9007</td>
</tr>
<tr>
<td>MDA/CAT</td>
<td>-0.8752</td>
<td>NO/CAT</td>
<td>-0.9013</td>
<td>ROS/CAT</td>
<td>-0.8825</td>
</tr>
<tr>
<td>MDA/SOD</td>
<td>-0.9035</td>
<td>NO/SOD</td>
<td>-0.9247</td>
<td>ROS/SOD</td>
<td>-0.9176</td>
</tr>
<tr>
<td>MDA/NO</td>
<td>+0.8990</td>
<td>MDA/ROS</td>
<td>+0.9317</td>
<td>NO/ROS</td>
<td>+0.9301</td>
</tr>
</tbody>
</table>

* $P \leq 0.05$

Correlation studies

Table (9) displays a correlation analysis of the parameters of the antioxidant response systems (antioxidant vitamins, antioxidant enzymes, and GSH fractions) and oxidants (ROS, MDA, and NO) in diabetes rats treated with GLE. Significant variations between oxidants and antioxidant defense system parameters were discovered when all treatments were included in the statistical examination. The concentration of glutathione portions (GSH, $r^2 = -0.9361$ and GSSG, $r^2 = -0.9136$) in plasma, antioxidant vitamins ($r^2 = -0.88690$), vitamin C ($r^2 = -0.8151$), and vitamin E ($r^2 = -0.8943$), antioxidant enzymes in red blood cells (rBCs) ($r^2 = -0.9046$), GSH-Px ($r^2 = -0.9476$), GSTh/Rd ($r^2 = -0.9129$), CAT ($r^2 = -0.8752$) and SOD ($r^2 = -0.9035$)], and MDA levels within plasma were found to be strongly negatively significant ($p \leq 0.05$). All of these parameters showed the same behavior, or correlations/relationships, with the quantity of ROS and NO in plasma. These associations demonstrate that it would be challenging to detect elevated levels of ROS, MDA, and NO in diabetic rats if there had been no change in the specifications of their antioxidant defense systems. In a related study, Böhm et al. (1997) found that β-tocopherol and β-carotene work together to inhibit lipid peroxidation, which in turn increases MDA in certain model systems. Furthermore, Shalaby (2014) found that relatively low levels of antioxidant vitamins and enzymes were linked to high levels of lipid peroxidation, or MDA, in the plasma of diabetic rats. Likewise, Elhassaneen et al. (2021a) reported that in diabetic rats fed Catharanthus roseus extracts, substantial variations were observed between plasma MDA and both GSH fractional ones and antioxidant enzymes. These extracts frequently include a large number of the bioactive substances present in the extract under investigation, GLE.

CONCLUSION

The lifelong illness known as diabetes results in hyperglycemia, or elevated blood sugar levels. A high risk of complications is associated with oxidative stress, which is one of the many factors which contribute to the physiology of diabetes in addition to hyperglycemia. Results from this investigation have shown how effective GLE is at reducing hyperglycemia and oxidative stress linked to diabetes in rats with diabetes that have been exposed to alloxan. Given that GLE contains high concentrations of several bioactive substance categories, it is possible to explain all of these ameliorating effects. By a variety of mechanisms of action, such as 1) growing GSH synthesis, 2) raising redox status (GSH/GSSG ratio), 3) stimulating antioxidant enzyme activity in RBCs (GSH-Px, GSH-Rd, CAT, and SOD), 4) enhancing levels of antioxidant vitamins (A, C, and E) in plasma, 5) inhibiting nitric oxide synthase, which reduces NO formation, and 6) inhibiting lipid oxidation, which reduces MDA formation in plasma. In light of the suppression of oxidative stress, one of the causes of type-2 diabetes and its related health risks, these findings advance the use of Ganoderma lucidum extracts for the prevention and/or treatment of the condition.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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المملوكة العربي

التدخل الغذائي باستخدام الفطر الريشي (جانوديرما لوسيدم) يقلل من الإجهاد التأكسدي ويترز آثاره
الدفاع المضادة للأكسدة للكريات الدم الحمراء والسيروم في الفئران المصابة بمرض السكري المستحث
بالألكسان

يوسف عبد العزيز الحسناني، سارة سمير عقاد، إيمان بدوى محرم

مرض السكري هو مرض أيضي يسبب ارتفاع مستوي السكر في الدم. في الح_you_، هناك العديد من العوامل الأخرى التي تلعب دورًا كبيرًا في التسبب في هذا المرض. الإجهاد التأكسدي الذي يؤدي إلى ارتفاع مخاطر حدوث مضاعفات هذا المرض. لذلك أجريت هذه الدراسة لمعرفة مدى فاعلية التدخل بمستخلص الإيثانول من الفطر الريشي. كان هناك تحسن معنوي (P≤0.05) في أنظمة الدفاع المختلفة لمستويات الإجهاد التأكسدي في كل من البلازما والدم. في النهاية، تأثرت النتائج هذه الدراسة أسانًا لاستخدام مستخلصات الفطر الريشي. في الدم، وسادي مستمع، السوبراءكسيد ديمبيوتيرز، الكاتالاز. وفي النهاية، تأثرت النتائج هذه الدراسة أسانًا لاستخدام مستخلصات الفطر الريشي. في الدم، ومع مراقبة وعلاج مرض السكري من النوع الثاني، بلغ جينية تأكسدي، والدم، والكبد. والمضاعفات الناتجة عنه.

الكلمات المفتاحية: ارتفاع السكر في الدم، الجلوتاثيون، انخفاض ارتفاع السكر في الدم، الجلوتاثيون، انخفاض ارتفاع السكر في الدم