ALEXANDRIA

SCIENCE EXCHANGE JOURNAL

VOL. 45 APRIL- JUNE 2024

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

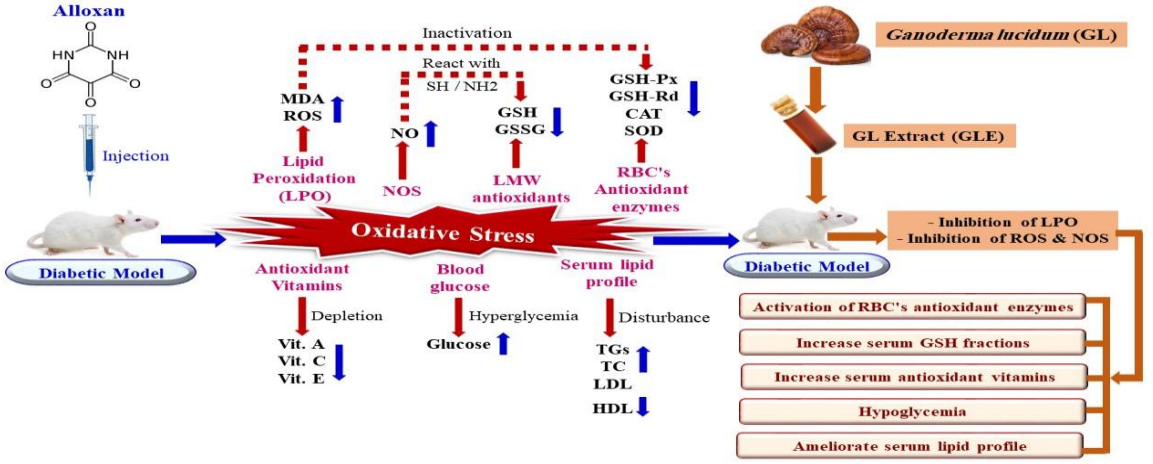
Yousif A. Elhassaneen [1] *, Sarah S. Aggad [2] and Eman B. Meharm[3]

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



DOI: 10.21608/asejaiqjsae.2024.354612

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

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

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

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

Received April 10, 2024, Accepted, May 15, 2024.

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 pro-oxidant/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 (O^{2-}) and hydrogen peroxide (H_2O_2) 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

(Wasser, 2005; Stojkovic *et al.* 2014 and Gharib *et al.*, 2022). Several pharmacological effects including immunomodulating, anti-atherosclerotic, analgesic, anti-inflammatory, chemo preventive, 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-Misryia 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-Ghomhorya 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 Schematzu 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 spherosorb 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 ±4.3g 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 Semiautomatic 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 Rules 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 ^{0}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 descried by Fossati & Prenape (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 ddetermined 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 NO_2 and NO_3 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 ($p \leq 0.05$) 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 ($p \leq 0.05$) 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 PER 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

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

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

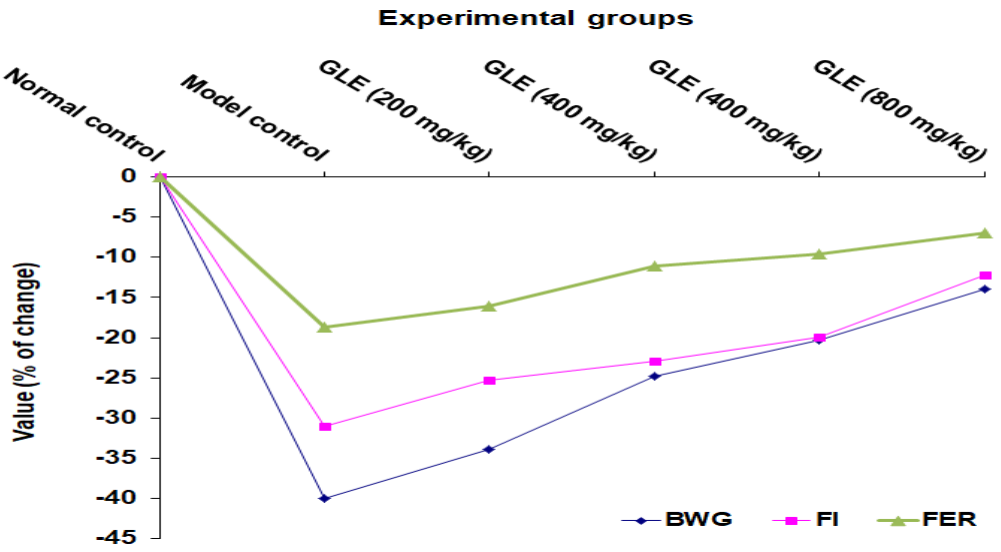


Fig. 1. Effect of GLE intervention on BWG, FI, and FER of diabetic rats

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 ($p \leq 0.01$) 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 ($p \leq 0.05$) 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 (Pusztai *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); Aly *et al.* (2017); Elbasouny *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 ($p \leq 0.05$) 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 ($p \leq 0.05$) 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 ($p \leq 0.05$) 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 ($p \leq 0.05$) 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

Group	Serum glucose conc.	
	Value (mg/dL)	Percent of normal control (%)
Normal control	92.67± 5.12^{e}	--------
Model control	311.24± 17.46^{a}	235.86
GLE intervention (200 mg/kg b w)	294.26± 8.56^{b}	217.54
GLE intervention (400 mg/kg b w)	268.34± 10.54^{c}	189.57
GLE intervention (600 mg/kg b w)	210.17± 10.37^{d}	126.79
GLE intervention (800 mg/kg b w)	192.78± 14.71^{d}	108.03

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

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

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

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

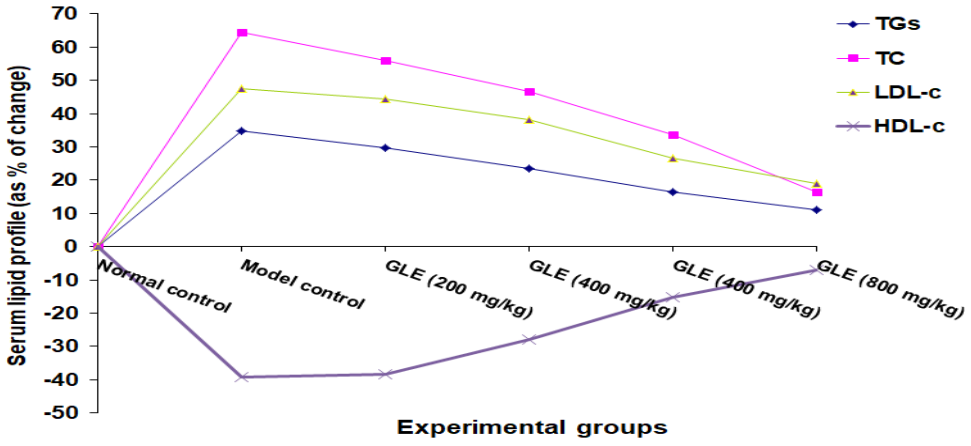


Fig.2. Effect of GLE intervention on serum lipid profile of diabetic rats

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 ($p \leq 0.05$). The levels of GSH and GSSG substantially ($p \leq 0.05$) 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

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

Each value represents mean ± SD (n=6 rats). Means on the same column with superscript letters indicate a significant difference at $P \leq 0.05$. Data for the experimental groups are as under Table 1. GSH; Reduced glutathione; GSSG: oxidized glutathione.

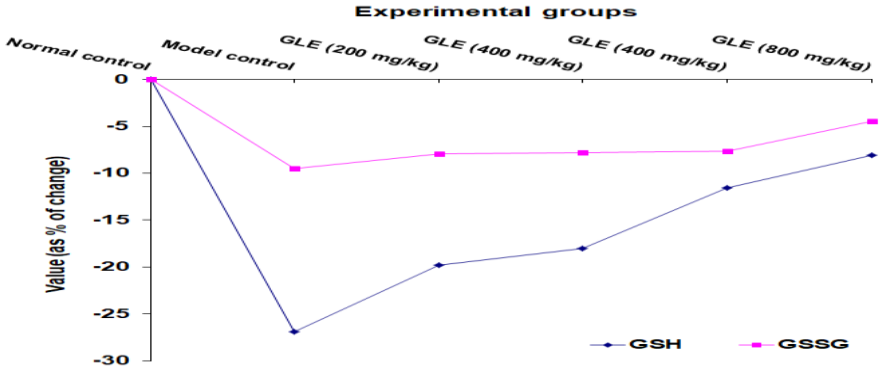


Fig. 3. Effect of GLE intervention on glutathione fractions of diabetic rats

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 ($p \leq 0.05$). 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 ($p \leq 0.05$) lower in the alloxan-treated rats than in the control group. Increases in GSH-Px, GSH-Rd, CAT, and SOD levels are significant ($p \leq 0.05$) 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. $O2^{\cdot -}$ is converted by SODs to H_2O_2, 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 H_2O_2 after the GSH reduces the Se. In normal cells, the nine GSH/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 + $H^+ \rightarrow$ 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

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

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

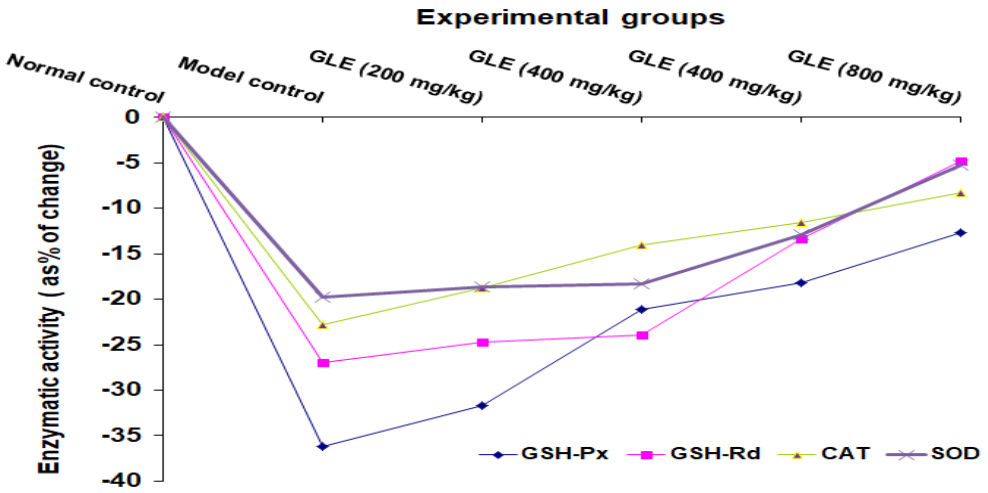


Fig. 4. Effect of GLE intervention on erythrocytes antioxidant enzyme activities of diabetic rats

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 ($p \leq 0.05$) 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 ($p \leq 0.05$) 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 influenced by dose increase. Furthermore, to red blood cells, plasma is plenty in naturally occurring antioxidant substances like β-carotenoids and lipid-soluble vitamins (like A and E) (Wohaieb 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 ($p \leq 0.05$) in ROS concentration in serum, 34.07%. In comparison to the normal control animals, the rates of ROS were significantly ($p \leq 0.05$) 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.*,

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

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

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

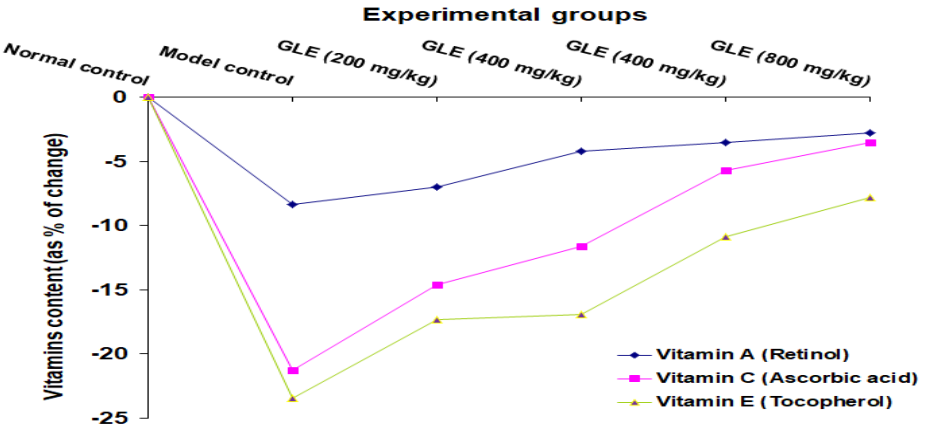


Fig. 5. Effect of GLE intervention on serum antioxidant vitamins of diabetic rats

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

Group	ROS conc.	
	Value (U/mL)	Percent of change (%)
Normal control	59.34± 4.98 [c]	------
Model control	79.56± 7.67[a]	34.07
GLE intervention (200 mg/kg b w)	78.09± 7.45 [a]	31.60
GLE intervention (400 mg/kg b w)	75.45± 5.98 [ab]	27.15
GLE intervention (600 mg/kg b w)	69.37± 8.11 [b]	16.90
GLE intervention (800 mg/kg b w)	65.71± 5.55 [bc]	10.74

Each value represents mean ± SD (n = 6). Different superscript letters on the same column indicate significant difference ($P \leq 0.05$). 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 ($p \leq 0.05$) 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 ($p \leq 0.05$) decreased MDA and NO_2 levels. With the GLE intervention, the rate of decline of oxidants (MDA and NO_2) 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 nitrate, 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

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

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

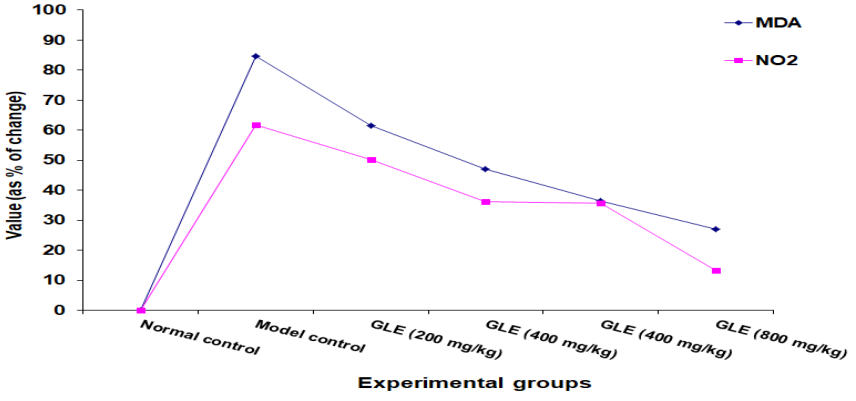


Fig. 6. Effect of GLE intervention on serum oxidants of diabetic rats

Table 9. Correlation between oxidants and antioxidant defense systems parameters in diabetes rat's intervention with GLE

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

** $P \leq 0.05$*

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, r2 = -0.9361 and GSSG, r2 = -0.9136) in plasma, antioxidant vitamins (r2 = -0.88690), vitamin C (r2 = -0.8151), and vitamin E (r2 = -0.8943), antioxidant enzymes in red blood cells (rBCs) (r2 = -0.9046), GSH-Px (r2 = -0.9476), GSH-Rd (r2 = -0.9129), CAT (r2 = -0.8752) and SOD (r2 = -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.* (2021 a) 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.

ACKNOWLEDGEMENTS

The authors appreciate the kind technical assistance provided by Rawan Shawky, Reem Boraey and Mona Elhefny, graduate students in the Department of Nutrition and Food Science, Faculty of Home Economics, Minoufia University, Shebin El-Kom, Egypt.

REFERENCES

Abd Elalal, N., S.A. Elsemelawy and Y.A. Elhassaneen. 2022. Potential effects of wild milk thistle (*Silybum marianum* L.) seed extract intervention on oxidative stress induced by busulfan drug in different organs of rats. *International J. of Healthcare and Medical Scie.* 8(3): 19-34. [DOI: 10.32861/ijhms.83.19.34].

Abd El-Rahman, N. 2021. Potential effect of olive and mango leaves in diabetic rats induced by alloxane. MSc. Thesis in Nutrition and Food Sci., Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt.

Aboraya, A. O., Y. A. Elhassaneen and O. M. Nassar. 2022. Reishi Mushroom (*Ganoderma lucidum*) intervension improves lipids profile and paraoxonase/arylesterase activitiesin serum as well as enhances haemostatic effects in streptozotocin-induced diabetic rats. Alexandria Sci. Exchange J. 43(4): 593-608. [DOI: 10.21608/asejaiqjsae.2022. 271965].

Aebi, H. 1974. Catalase. In: Bergmeyer HU. Ed. Methods of enzymatic analysis. New York, London: Academic Press. 673-677.

AIN, American Institute of Nutrition. 1993. Purified diet for laboratory Rodent, Final report. J. Nutrition. 123:1939-1951.

Alberici, L. C., A. E. Vercesi and H. C. Oliveira. 2011. Mitochondrial energy metabolism and redox responses to hypertriglyceridemia. J. Bioenerg. 43: 19–23.

Almutairiu, F.M. 2020. Potential effects of phyto-bioactive and aversive on obesity and its complications in rats". M.Sc. Thesis in Nutrition and Food Sci., Faculty of Specific Education, Benha University, Benha, Egypt.

Aly, A., G.M. Elbassyouny and Y.E. Elhassaneen. 2017. Studies on the antioxidant properties of vegetables processing by-products extract and their roles in the alleviation of health complications caused by diabetes in rats. Proceeding of the 1st International Conference of the Faculty of Specific Education, Kafrelsheikh University, "Specific Sciences, their Developmental Role and Challenges of Labor Market" 24-27 October 1-24, Sharm ElSheikh, Egypt.

Aviram, M. and J. Vaya. 2013. Paraoxonase 1 activities, regulation, and interactions with atherosclerotic lesion, *Current Opinion in Lipidology*. 24(4): 339–344.

Beck-Nielsen, H. 2002. Insulin resistance: organ manifestations and cellular mechanisms. Ugeskr. Laeger. 15; 164(16): 2130-5.

Bedard, K. and K. Krause. 2007. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiological Reviews. 1: 245–313.

Bedawy, O. 2008. Relationship between phyto-sulphur compounds and lipid of blood in experimental animals. M. Sc. Thesis in Nutrition and Food Sci., Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt.

Birben, E., U. M.Sahiner, C. Sackesen, S. Erzurum and O. Kalayci. 2012. Oxidative Stress and Antioxidant Defense. WAO J. 5: 9-19.

Böhm, F., R. Edge, E.J. Land, D.J. McGarvey and T.G. Truscott. 1997. Carotenoids enhance vitamin E antioxidant efficiency. *J Am Chem Soc.* 119: 621-622.

Buege, J.A. and S.D. Aust. 1978. Microsomal lipid peroxidation in Packer L., (ed), Methods in enzymology, New York, NY, Academic. 52: 302 - 310.

Cao, H. 2014. Adipocytokines in obesity and metabolic disease, *J. of Endocrinology*. 220(2): T47–T59.

Caro, J. F. 1990. Effect of glyburide on carbohydrate metabolism and insulin action. *Am. J. Med.* 89 (20): 17S-24S.

Champe, C.P. and A.R. Harvey. 1994. Biochemistry. 2nd edition, J.B. Lippincott Company, Philadelphia, USA

Chang, S. T. and J. A. Buswell. 2008. Safety, quality control and regulational aspects relating to mushroom nutriceuticals. *Proc. 6th Intl*. Conf. *Mushroom Biology and Mushroom Products*. pp.188–95. Krefeld, Germany.

Chapman, D.G., R.Castilla and J.A. Champbell. 1959. Evaluation of protein in food. I.A Method for the determination of protein efficiency ratio. Can. *J.* Biochemistry on Liver Disorder Initiation by carbon Tetra Chloride. M. Sc Thesis, Faculty of Home Physiology. 37: 679-686.

Cosentino, F., K.Hishikawa, Z.S. Katusic and T.F. Liischer. 1997. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation.* 96:25-28.

Curtis, J.M., P.A. Grimsrud and W.S. Wright. 2010. Down regulation of adipose glutathione S-tansferase A4 leads to increased protein carbonylation, oxidative stress, and mitochondrial dysfunctio. Diabetes. 59 (5): 1132–1142.

Davies, M.J. and R.T. Dean. 1997. Radical-Mediated Protein oxidation. From chemistry to Medicine. Oxford, England: Oxford University Press.

Donath, M. Y. and S. E.Shoelson. 2011. Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.* 11: 98–107. doi: 10.1038/nri2925

Dos Santos, J.M., S.Tewari and R.H.Mendes. 2019. The Role of Oxidative Stress in the Development of Diabetes Mellitus and Its Complications, *J. of Diabetes Research*. Article ID 4189813.

Elbasouny, G., N.Shehata and Y. Elhassaneen. 2019. Feeding of some selected food industries by-products induced changes in oxidants/antioxidant status, lipids profile, glucose and immunological parameters of blood obese rats. The 6th Scientific and 4th International Conference "The Future of Specific Education and people with Special Needs in Light of the Concept of Quality ", Faculty of Specific Education, Ain Sokhna University, El-Ain El-Soghna, Egypt. 24-26 February.

El-Gamal, N. 2020. Studies on the antioxidant activities of brown algae and their effects on obesity and osteoporosis in rats. Ph.D. Thesis in Nutrition and Food Sci., Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt.

Elhassaneen, Y. A., A. E. El-Khamisy, N. F. Salem and E. M. El-Hawary. 2023. Possible Mechanisms Underlying the Therapeutic Effects of Brown Algae (Sargassum subrepandum) for Oxidative Stress in Diabetic Rats. *American J. of Food and Nutrition.* 11(3):100-111. [DOI:10.12691/ajfn-11-3-5]

Elhassaneen, Y. A., S. A. Khader, M. A. Gharib and Y. E. Abd-ElAziz. 2024. Possible Protective Roles of Poinciana (*Delonix regia*) Seeds Against Carbon Tetrachloride-induced Biochemical and Histological Disorders in Rat Liver. *American J. of Medical Sciences and Medicine*. 12(1):1-15. [DOI:10.12691/ajmsm-12-1-1].

Elhassaneen, Y., A. Abdel Rhman and N. Hussin. 2021a. The Potential Effects of Psyllium Seeds and its Husks (*Plantago ovata*) on Diabetic Rats. *J. of Home Economics*. 31 (2): 36-48. [DOI: 10.21608/mkas.2021.181265].

Elhassaneen, Y. and A. Salem. 2014. Biochemical/Nutritional Studies on some Obesity Cases in Egypt. *J. of Home Economics*. 24(1): 121-137.

Elhassaneen, Y., S. Goarge, R. Sayed and M. Younis. 2016a. Onion, orange and prickly pear peel extracts mixed with beef meatballs ameliorate the effect of alloxan induced diabetic rats. *American J. of Pharmacology and Phytotherapy*. 1 (1): 15-24. [doi: 10.11648/j.ajpp.20160101.14].

Elhassaneen, Y., M.El-Dashlouty and N. El-Gamal. 2020. Effects of brown algae (*Sargassum subrepandum*) consumption on obesity induced changes in oxidative stress and bone indices. *J. of Home Economics. 30(4):*687-608.

Elhassaneen, Y., S.Hassab El-Nabi, M.Mahran, A. Bayomi and E.Badwy. 2022a. Potential Protective Effects of Strawberry (*Fragaria Ananassa*) Leaves Against Alloxan Induced Type 2 Diabetes in Rats: Molecular, Biological and Biochemical Studies. *Sumerianz J. of Biotechnology*, 5(1): 1-15. [DOI: https://doi.org/10.47752/sjb.51.1. 15] .

Elhassaneen, Y., A. Nasef and N.Abdel Rhman. 2021b. Potential Effects of Olive and Mango Leaves on Alloxan Induced Diabetes Complications in Rats. *J.* of Home Economics. 31(2):49-62.

Elhassaneen,Y., S.Ragab, M.Thoraya, E.Azza and A. Abeer. 2012. Effect of Sweet Violet (*Viola odorata* L.) Blossoms Powder on Liver and Kidney Functions as well as Serum Lipid Peroxidation of Rats Treated with Carbon Tetrachloride. *The J. of American Sci.* 9 (5):88-95.

Elhassaneen, Y., S.Ragab, A.Abd El-Rahman and S.Arafa. 2021c. Vinca (*Catharanthus roseus*) Extracts Attenuate Alloxan-Induced Hyperglycemia and Oxidative Stress in Rats. American *J.* of Food Sci. and Technology. 9 (4): 161-172.

Elhassaneen, Y., S.Sayed Ahmed, S.Elwasef and S.Fayez. 2022b. Effect of brown algae ethanolic extracts consumption on obesity complications induced by high fat diets in rats. *Port Saied Specific Research J. (PSSRJ).* 15(1): In Press. [DOI:1 10.21608/pssrj.2021.98769.1148].

Elhassaneen, Y., K.Seham and M.Salman. 2019. Effect of Some Plant Parts Powder on Obesity Complications of Obese Rats. *J. of Home Economics*. 29(1): 83-106.

Elhassaneen, Y., M.Mohamed and H.Hassan. 2014. The effect of some food products mixed with plant parts on blood sugar levels of rats. 3rd International-17 th Arab Conference of Home Economics "Home Economics in the Service of Sci., Industry and Society Issues" 9-11 September, 2014, Faculty of Home Economics. Minoufiya University, Egypt. *J. of Home Economics (Special issue).* 24(4): 85-109.

Elhassaneen, Y., S.Ragab and A.Saleh. 2015. Effect of selected plant parts extracts on liver injuries induced by CCl_4 *in vitro*. *Pyrex J. of Medicinal Plant Research*. 2(2): 8-20.

Elhassaneen, Y.A. 2004. The effect of charcoal broiled meat consumption on antioxidant defense system of erythrocytes and antioxidant vitamins in plasma. *Nutrition Research*. 24(6):435-446.

Elhassaneen, Y.A. and A.A. Abd El-Moaty. 2003. Blood oxidant and antioxidant status in rats feeding with insect-infested wheat flour. *Pakistan J. of Biological Sci.* 6(15):1354-1360.

Elhassaneen,Y., S. Ragab and M.Salman. 2016b. The Potential Effects of Reishi Mushroom (*Ganoderma lucidum*) Consumption on Bone Health Indices and Serum Minerals Profile Disorders Induced by CCl4 on Rats. *Pyrex J. of Medicinal Plant Research*. 2 (1):1-7.

Elhassaneen,Y.A. 1996. Biochemical and technological studies in the pollution of fish with pesticides and polycyclic aromatic hydrocarbons. Ph.D. Thesis, Faculty of Agriculture, Mansoura University, Mansoura, Egypt

Elmaadawy, A., R.M. Arafa and Y. A. Elhassaneen. 2016. Oxidative Stress and antioxidant defense systems status in obese rats feeding some selected food processing by-products applied in bread. *J. of Home Economics*. 26(1):55-91.

Elsemelawy, S., M.Gharib and Y.Elhassaneen. 2021. Reishi Mushroom (Ganoderma lucidum) Extract Ameliorate Hyperglycemia and Liver/Kidney Functions in Streptozotocin-induced Type 2 Diabetic Rats. *Bulletin of the National Nutrition Institute of the Arab Republic of Egypt*. 57: 74-107.

Epler, K.S., R.G. Zeigler and N.E. Craft. 1993. Liquid chromatographic method for the determination of carotenoids, retinoids and tocopherols in human serum and in food. *J Chromatog.* 619: 37–48.

Erel, O. 2005. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 38:1103–11.

Esterbauer, H., J.Gebicki, H.Puhl, and G.Jurgens. 1992. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Rad Biol Med*. 13: 341-390.

Evans, J. L., I. D.Goldfine, B. A.Maddux and G. M.Grodsky. 2003. Are oxidative stress—activated signaling pathways mediators of insulin resistance and b-cell dysfunction? *Diabetes*. 52:1–8.

Fossati, P. and L. Prenape. 1982. Serum triglycerides determined colorimeterically with enzyme that produce hydrogen peroxide. Clin. Chem. 28: 2077-2080.

Galinier, A., A. Carriere and Y. Fernandez. 2004. Adipose tissue proadipogenic redox changes in obesity, The *J.* of Biological Chemistry. 281(18):12682–12687.

Gao, Y., J.Lan, X.Dai, J.Ye and S.H.Zhou. 2004. A phase I=II study of Ling Zhi *mushroom Ganoderma lucidum. (*W. Curt.: Fr.) Lloyd (*Aphyllophoromycetideae*) extract in patientswith type II diabetes mellitus. *Int. J. Med.Mushrooms.* 6 (1): 1-8.

Gharib, M. A., H.A. Radwan and Y. A. Elhassaneen. 2022. Nutrients and Nutraceuticals Content and *in Vitro* Biological Activities of Reishi Mushroom (Ganoderma lucidum) Fruiting Bodies. *Alexandria Sci. Exchange J.* 43(2): 301-316. [DOI: 10.21608/asejaiqjsae.2022. 245271].

Goldin, A., J. A.Beckman, A. M.Schmidt and M. A.Creager. 2006. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 114:597–605.

Górnicka, M., M.Drywień, J.Frąckiewicz, B.Dębski and A.Wawrzyniak. 2016. Alpha-Tocopherol May Protect Hepatocytes Against Oxidative Damage Induced by Endurance Training in Growing Organisms. *Adv Clin Exp Med.* 25: 673-679.

Halliwell, B. 1991. Reactive oxygen species in living systems: Source, biochemistry and role in human disease. *American J. of Med.* 91:14s-21s.

Halliwell, B. and J.M. Gutteridge. 1985. Free radicals in biology and medicine. Clarendon Press. Oxford. UK.

Hamzawy M.A., E.S.El-Denshary, F.A.Mannaa and M.A. Abd Elwahhab. 2013. Dietary supplementation of *Calendula officinalis* counteracts the oxidative stress and liver damage resulted from aflatoxin. *ISRN Nutrition*. 9(10):5402-538427.

Hung, S.S., Y.C. Cho and S.J.Slinger. 1980. Hight performance liquid chromatographic determination of alpha-tocopherol in fish liver, *J. Assoc. Off. Anal. Chem.* 63(4):889- 893.

ICSH, International Committee for Standardization in Haematology. 1979. Recommended methods for red cell enzyme analysis. *British J. of Haematology*. 35: 331-340.

IDF, International Diabetes Federation. 2021. IDF Diabetes Atlas,10th edition, Brussels, Belgium.

Jacob, T.D., M.K.Morrell, S.Manzi, J.B.Ochoa, V.Verdile, A.O.Udekwu, S.A.Berceli, R.L.Simmons, and A.B.Peitzman. 1992. Nitric oxide: Implications for drug research. pp.28, IBC, South Natick, MA.

Jung. J. Y., L.Yeni, S.Min, Y. K. Ji and K.Oran. 2011. Onion peel extracts ameliorate hyperglycemia and insulin resistance in high fat diet/streptozotocin-induced diabetic rats, *Nutrition & Metabolism.* 8:18:2-8.

Konstantinos, P., Maciej, B., Eleni, B. and Manfredi, R. (2018). Complications of Diabetes 2017. *Journal of Diabetes Research* 2018:1-4

Lazarow, A. and B.Palay. 1954. Experimental Diabetes and its relation to the Disease" Asymposium. *Black wells scientific Publication.* 14: 66 – 69.

Lenzen, S. 2008. The mechanisms of alloxan- and streptozotocin-induced Diabetes. *Diabetologia*. 51:216–226.

Leskosek, I., D.Sasa, N.Lakic and M.Niksic. 2010. Ganoderma lucidum Medical mushroom as a raw material for beer with enhanced functional properties. *Food Research International.* 43(9):2262-2269.

Li, J.M. and A. M.Shah. 2003. ROS generation by nonphagocytic NADPH oxidase: potential relevance in diabetic nephropathy. *J. Am. Soc. Nephrol.* 14(Suppl. 3):S221–S226.

Liu, K. C., S. F.Phounsavan, R. L.Huang, C.Liao, S. Y. Hsu and K. J. Wang. 1998. Pharmacological and liver functional studies on mycelium of *Ganoderma lucidum*. *Chin Pharm J.*40:21–9.

Liu, Z., J.Xing, S.Zheng, R.Bo, L.Luo, Y.Huang, Y.Niu, Z.Li, D.Wang, Y.Hu, J. Liu and Y.Wu. 2016. Ganoderma lucidum polysaccharides encapsulated in liposome as an adjuvant to promote Th1-bias immune response. Carbohydr. *Polym.* 142:141-148.

Lopes-Virella, M.F., S.Stone, S.Ellis and J.A.Collwell. 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin. Chem.* 23(5): 882-886.

LU, S.C. 1999. Regulation of hepatic glutathione synthesis: current concepts and controversies, *FASEB J.* 13:1169–1183.

Ma, X., Z.Chen, L.Wang, G.Wang, Z.Wang, X.Dong, B.Wen and Z. Zhang. 2018. The Pathogenesis of Diabetes Mellitus by Oxidative Stress and Inflammation: Its inhibition by Berberine. Front. *Pharmacol.* 9:782.

Mahran, M.Z. and Y.A. Elhassaneen. 2023. Attenuation of Benzo[a]pyrene-Induced Oxidative Stress and Cell Apoptosis in Albino Rats by Wild Milk Thistle (Silybum Marianum L.) Seeds Extrac. *Egypt. J. Chem.* 66 (SI: 13): 1671 - 1687. [DOI: 10.21608/EJCHEM.2023.214010.8042].

Malivindi, R., V.Rago, D.De Rose. 2018. Influence of all-trans retinoic acid on sperm metabolism and oxidative stress: Its involvement in the physiopathology of varicocele-associated male infertility. *J Cell Physiol.* 233:9526-9537.

Manahan, S.E. 1989. Toxicological chemistry: A guide to toxic substances in chemistry, PCR press, New york.

Mathe, D. 1995. Dyslipidemia and diabetes: animal models. *Diabete. Metab.* 21(2):106-111.

Mayhan, W.G. (1997). Superoxide dismutase partially restores impaired dilatation of the basilar artery during diabetes mellitus. *Brain Res*, 760:204-209.

McCord, J.M., B.B. Keele and I.Fridovich Jr. 1976. An enzyme-based theory of obligateanaerobiosis: the physiological function of superoxide dismutase, *Proc. Natl. Acad. Sci.* U.S.A. 68:1024–1027.

McFarris, M.W. and D.J. Reed. 1987. HPLC of thiols and disulfides: dinitrophenol derivatives. *Methods Enzymol*. 143:101–109.

McKenna, D.J., K.Jones and K.Hughes. 2002. Reishi Botanical Medicines. The Desk reference for Major Herbal Supplements, 2nd Ed.; The Haworth Herbal Press: New York, London, Oxford. *pp*. 825–855.

Mett, J. and U.Müller. 2021. The medium-chain fatty acid decanoic acid reduces oxidative stress levels in neuroblastoma cells. *Sci Rep*. 11:6135.

Miranda, K.M., M.G. Espey and D.A.Wink. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric oxide.* 5(1):62-71.

Misko, T., R.Schilling, D.Salvemini, W.Moore and M.Currie. 1993. A Fluorometric assay for the measurement of nitrite in biological samples. *Analytical Biochemistry*. 214: 11-16.

Mizuno, T. 1995. Reishi, Ganoderma lucidum and Ganoderma tsugae: bioactive substances and medicinal effects. *Food Rev. Int*. 11 (1):151–166.

Moeslinger, T., M.Brunner and G.Spieckermann. 1994. Spectrophotometric determination of dehydroascorbic acid in biological samples. *Analytical Biochemistry*. 221: 290 - 296.

Morresion, G. and L.Hark. 1999. Medical Nutrition and Disease. Second Edition. Black Willy, USA.

Musallam, L., C.Ethier, P.S.Haddad, F.Denizeau and M. Bilodeau. 2002. Resistance to Fas-induced apoptosis in hepatocytes: role of GSH depletion by cell isolation and culture. *Am J Physiol Gastrointest Liver Physiol.* 283: G709–G718.

NRC, National Research Council. 1996. Guide for the Care and Use of Laboratory Animals Washington: National Academy Press.

Olorunnisola, O., A.Ajayi, L.Okeleji, A.Oladipo and J.Emorioloye. 2019. Vitamins as Antioxidants. *J. of Food Science and Nutrition Research*. 2: 214-235.

Ozougwu, J.C. 2011. Anti-diabetic effects of Allium cepa (onions) aqueous extracts on alloxan-induced diabetic Rattus novergicus. *J*. of Medicinal Plants Research. 5(7):1134-1139.

Padayatty, S. J., A. Katz and Y.Wang. 2003. Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. *J. of the American College of Nutrition*. 22:18-35.

Pieper, G.M. and W.Siebeneich. 1997. Diabetes-induced endothelial dysfunction is prevented by long-term treatment with the modified iron chelator, hydroxyethyl starch conjugated-deferoxainine. *J. Cardiovasc Pharni*. 30:734-738.

Prokopowicz, A., A.Sobczak, M.Szuła, E.Anczyk, J.Kurek, Z.Olszowy, M.Radek, N.Pawlas, P.Ochota and I.Szołtysek-Bołdys. 2013. Effect of occupational lead exposure on - and -tocopherol concentration in plasma. Occup Environ. Med. 70:365–371.

Pusztai, P., J.Prechl, A. Somogy, E.Szaleczky. and J.Feher. 1996. Experimental models in research of the pathomechanism of diabetes mellitus. *Orv. Hetil.* 137(34):1865-1869.

Richmod, W. 1973. Determination of cholesterol by enzymatic colorimetric method. Clin. Chem. 19: 1350-1356.

Sayed Ahmed, S. 2016. Nutritional and technological studies on the effect of phytochemicals on obesity injuries and their related diseases by using experimental animals. Ph.D. Thesis in Home Economics (Nutrition and Food Sci.), Faculty of Specific Education, Port Said University, Egypt.

Sayed, K. M. 2020. Chemical and Technological Studies on some Food Products Supplementing with Cortices Fruit. Ph.D. Thesis, Faculty of Specific Education, Minia University, Minia, Egypt.

Sayed-Ahmed, S. , V.Shehata and Y.Elhassaneen. 2020. Potential Protective Effects of *Ganoderma lucidum* Powder against Carbon Tetrachloride Induced Liver Disorders in rats: Biological, Biochemical and Immunological Studies, Egypt. *Bulletin of the National Nutrition Institute of the Arab Republic of Egypt.* 56(2): 99-132. [DOI: 10.21608/bnni.2020.196206].

Schriewer, H., U. Kohnert and G.Assmann. 1984. Determination of LDL cholesterol and LDL apolipoprotein B following precipitation of VLDL in blood serum with phosphotungstic acid/MgCl2. *J. Clin Chem Clin Biochem.* 22(1):35-40.

Shalaby, H. 2014. The effect of some food products mixed with plant parts on blood sugar levels of rats " Ph. D. Thesis in Nutrition and Food Sci., Faculty of Home Economics, Minoufiya University, Egypt.

Shalaby, H.S. and Y.A.E.Elhassaneen. 2021. Functional and health properties of yogurt supplemented with green tea or green coffee extracts and its effect on reducing obesity complications in rats. Alexandria Sci. Exchange J. 42(2):559-571.

Shamberger, R.J., T.L.Andreone and C.E. Willis. 1974. Antioxidants and cancer. IV. Malonaldehyde has initiating activity as a carcinogen. *J. Natr. Cancer Inst.* 53:1771.

Shin JiHyun, S.J., Y.S. Yang SooJin and L.Y. Lim YunSook. 2017. Gamma-tocopherol supplementation ameliorated hyper-inflammatory response during the early cutaneous wound healing in alloxan-induced diabetic mice. *Exp Biol Med (Maywood).* 242: 505-515.

Sies, H. 1985. Introductory remarks. In: Sies H (ed) Oxidative stress. Academic, London. pp 1–8.

Splittgerber, A.G. and A.L.Tappel. 1979. Inhibition of glutatione peroxidase by cadimium and other metal ions. *Arch Biochem Biophys.* 197:534–42.

Stadtman, E.R. 2004. Role of oxidant species in aging. *Curr Med Chem.* 11:1105–1112.

Steinberg, D., S.Parthasarathy, and T.E.Carew. 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl J. Med.* 320: 915-924.

Stojkovic, D.S., L.Barros, R.C.Calhelha, J.Glamo_clija, C.A.Ciri, L.J. van Griensven, C. M.Sokovi. and I.C. Ferreira. 2014. A detailed comparative study between chemical and bioactive properties of Ganoderma lucidum from different origins. Int. *J. Food Sci. Nutr*. 65, 42e47

Stroev, E.A. and V.G. Makarova. 1989. Laboratory Manual in Biochemistry, MIR Publishers, Moscow, USSR.

Taofiq, O., S.A.Heleno, R.C.Calhelha, M.J.Alves, L.Barros, A.M.González-Paramás, M.F.Barreiro and I.C.Ferreira. 2017. The potential of Ganoderma lucidum extracts as bioactive ingredients in topical formulations, beyond its nutritional benefits. *Food and chemical toxicology*. *108*:139-147.

Tietz, N.W. 1976. Fundamental of Clinical Chemistry. Philadelphia, W.B. Saunders. P. 243.

Tiwari, A.K. and J.M. Rao. 2002. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Sci.* 83(1):30-38.

Van Gaal, L.F., J. Vertommen and I.H. De Leeuw. 1998. The *in vitro* oxidizability of lipoprotein particles in obese and non-obese subjects. *Atherosclerosis.* 137: S39-S44.

Wachtel Galor, S., W. M. John, A. B. John and F. F. Iris. 2011. Ganoderma lucidum (Lingzhi or Reishi): A Medicinal Mushroom. In book: Herbal Medicine: Biomolecular and Clinical Aspects, Editors: Iris F. F. Benzie, Sissi Wachtel-Galor, Edition: 2nd. Chapter: Chapter 9, CRC Press

Wasser, S. 2005. Reishi or Ling Zhi (*Ganoderma lucidum*). Encyclopedia of Dietary Supplements. Marcel Dekker Inc., New York, NY,USA. 603-622.

WHO. 2023. "World Health Organization, diabetes", Rome, Italy, Available: https://www.who.int/ news-room/fact-sheets/detail/diabetes.

Wohaieb, S.A. and D.V. Godin. 1987. Alterations in free radical tissue defense mechanisms in streptozotocin induced diabetes in rat. Effects of insulin treatment. *Diabetes.* 36:1014-1018.

Yousuf, S.Y., S. Ambiger and D. Morkar. 2019. Serum Nitric Oxide and Plasma HbA1c Levels in Type 2 Diabetes Mellitus Patients. *J. of Clinical and Diagnostic Research*. 13(9):BC04-BC06.

الملخص العربي

التدخل الغذائى باستخدام الفطر الريشى (لجانوديرما لوسيدوم) يقلل من الإجهاد التأكسدي ويعزز أنظمة الدفاع المضادة للأكسدة لكريات الدم الحمراء والسيرم فى الفئران المصابة بمرض السكري المستحث بالألوكسان

يوسف عبد العزيز الحسانين، سارة سمير عقاد، ايمان بدوى محرم

مرض السكري هو مرض أيضي يسبب ارتفاع مستوى السكر في الدم إلى جانب ذلك فان هناك العديد من العوامل الأخرى التي تلعب دورًا كبيرًا في التسبب في هذا المرض مثل الإجهاد التأكسدي الذي يؤدي إلى ارتفاع مخاطر حدوث مضاعفات هذا المرض. لذلك أجريت هذه الدراسة لمعرفة مدى فاعلية التدخل بمستخلص الإيثانول للفطر الريشى (جانوديرما لوسيدوم) ضد الإجهاد التأكسدي في الخلايا الحية مع التركيز بشكل خاص على جوانبها الميكانيكية في علاج مرض السكرى. سجلت الجرذان الطبيعية (المجموعة الضابطة السالبة) 92.67 ملجم/ ديسيلتر لمستوى الجلوكوز في الدم والذي زاد بشكل معنوي ($p\leq0.01$) إلى 311.24 مجم/ ديسيلتر في الجرذان المعاملة بالالوكسان ، كما أدت التدخلات بالمستخلص الإيثانولى للفطر الريشى (GLE) بجرعات 200، 400، 600 إلى 800 ملجم / كجم من وزن الجسم في تغذية الفئران لمدة 28 يومًا إلى انخفاض معنوي ($p\leq0.05$)في مستويات الجلوكوز في الدم والتي سجلت 294.26، 268.34، 210.17، 192.78 مجم/ ديسيلتر على التوالي. كما اظهرت النتائج ان معدل النقص في جلوكوز الدم قد اعتمد على الجرعة التى تم التدخل من الـ GLE. كما تم تسجيل نفس السلوك بالنسبة للمؤشرات الحيوية لمستويات الإجهاد التأكسدي في البلازما والتى تشمل مستويات أنواع الأكسجين النشط (ROS) والمالونالدهيد (MDA) وأكسيد النيتريك (NO) ، وعلى العكس من ذلك كان هناك تحسن معنوى ($P\leq0.05$) في أنظمة الدفاع المختلفة لمضادات الأكسدة في كل من البلازما والتى تشمل الجزيئات النشطة بيولوجيًا مثل أجزاء الجلوتاثيون، والفيتامينات المضادة للأكسدة (أ، جـ، هـ) وكريات الدم الحمراء والتى تشمل الإنزيمات المضادة للأكسدة (الجلوتاثيون بيروكسيديز، الجلوتاثيون ريدكتيز، السوبرءاكسيد ديسميوتيز، الكاتاليز. وفى النهاية، توفر نتائج هذه الدراسة أساسًا لاستخدام مستخلصات الفطر الريشى (جانوديرما لوسيدوم) للوقاية وعلاج مرض السكري من النوع الثانىعن طريق قمع الإجهاد التأكسدي، والذي يمثل أحد أسباب المرض، والمضاعفات الناتجة عنه.

الكلمات المفتاحية: ارتفاع السكر في الدم، الجلوتاثيون، الإنزيمات المضادة للأكسدة، الفيتامينات المضادة للأكسدة، أنواع الأكسجين النشط، مالونالدهيد، اكسيد النتريك