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Therapeutic Potential of Milk Thistle (*Silybum marianum* L.) Seeds Extract in High-Fat Diet-Induced Obesity: Focusing on Oxidative Stress, Metabolic Dysfunctions, Neurological Complications and Liver Health

الإمكانات العلاجية لمستخلص بذور شوك الحليب (*Silybum marianum* L.) في السمنة الناتجة عن النظام الغذائي عالي الدهون: التركيز على الإجهاد التأكسدي واختلالات الأيض والمضاعفات العصبية وصحة الكبد

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Abstract:

Obesity, a major global health concern characterized by excessive body fat, is treatable; however, current pharmacological options are costly and often associated with side effects that reduce patient adherence. This has prompted interest in alternative therapies, especially those from natural sources, due to their affordability and fewer adverse effects. This study examines the effects of methanol extract from wild milk thistle seeds (*Silybum marianum* L., SME) on obesity and related complications in a high-fat diet (HFD)-induced rat model. Thirty-six adult male Sprague-Dawley rats were randomly assigned to six groups (n=6). Group 1 served as the normal control (standard diet), Group 2 as the model control (obese rats without treatment), and Groups 3–6 were treatment groups receiving SME orally at doses of 100, 200, 400, or 600 mg/kg/day for eight weeks. Obesity was induced using an HFD. After treatment, SME significantly ($p \leq 0.05$) reduced body weight, blood glucose, insulin, triglycerides, total cholesterol, and LDL-c, while increasing hepatic glutathione (GSH), antioxidant enzymes (GSH-Px, SOD, CAT), leptin, HDL-c, paraoxonase, and arylesterase activities compared to the model control. SME also decreased hepatic oxidative stress markers, including hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). Correlation analyses revealed improvements in insulin sensitivity, lipid metabolism, and oxidative stress, suggesting that SME mediates its anti-obesity effects through multiple biochemical pathways. These findings indicate that SME has significant potential as a natural therapeutic agent for managing obesity and related metabolic disorders, though further research is necessary to confirm its long-term efficacy and clinical applicability.

Keywords:

blood glucose, insulin, leptin, blood lipid components, glutathione, antioxidant enzymes, malonaldehyde.

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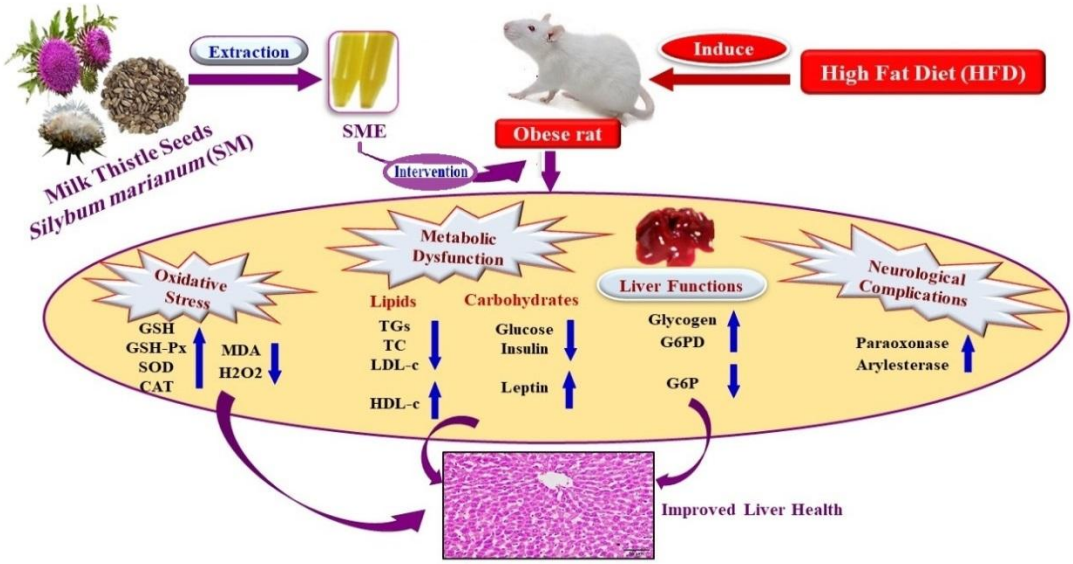
مستخلص البحث:

السمنة تُعد من أبرز القضايا الصحية العالمية، وتتميز بتراكم مفرط للدهون في الجسم، وهي حالة قابلة للعلاج. ومع ذلك، فإن الخيارات الدوائية الحالية غالبًا ما تكون مكلفة وترتبط بآثار جانبية تقلل من التزام المرضى بالعلاج، مما أثار الاهتمام بالبحث عن علاجات بديلة، خاصةً تلك المستخلصة من مصادر طبيعية نظرًا لتكلفتها المنخفضة وآثارها الجانبية المحدودة. تبحث هذه الدراسة في تأثير مستخلص الميثانول لبذور الشوك الحليب البري (SME) *Silybum marianum L.* على السمنة والمضاعفات المرتبطة بها باستخدام نموذج فئران تم تحفيز السمنة لديها بنظام غذائي عالي الدهون (HFD) . تم تقسيم 36 فأرًا بالغًا من ذكور الفئران عشوائيًا إلى ست مجموعات (6 فئران لكل مجموعة). مثلت المجموعة الأولى الضابطة السليمة (نظام غذائي قياسي)، والمجموعة الثانية كانت ضابطة نموذجية (فئران مصابة بالسمنة دون علاج)، أما المجموعات 3 إلى 6 فقد تلقت SME عن طريق الفم بجرعات 100، 200، 400، أو 600 ملج/كج يوميًا لمدة ثمانية أسابيع. أدى العلاج بـ SME إلى انخفاض معنوي ($p\leq0.05$) في الوزن، سكر الدم، الإنسولين، الدهون الثلاثية، الكوليسترول الكلي، وLDL-c، بالإضافة إلى زيادة في الجلوتاثيون الكبدي(GSH) ، وإنزيمات مضادة الأكسدةGSH-Px) ، SOD، (CAT، واللبتين، وHDL-c، ونشاط إنزيمي الباروكسيوناز والأريليستيراز مقارنة بالمجموعة الضابطة. كما قلل SME من مؤشرات الإجهاد التأكسدي في الكبد، مثل فوق أكسيد الهيدروجين (H_2O_2) والمالونداياألديهيد (MDA) . أظهرت تحليلات الارتباط تحسنًا في حساسية الإنسولين، واستقلاب الدهون، والإجهاد التأكسدي، ما يشير إلى أن SME يمارس تأثيراته المضادة للسمنة عبر مسارات بيوكيميائية متعددة. وتُظهر هذه النتائج أن لـ SME إمكانات علاجية واعدة كعامل طبيعي لإدارة السمنة والاضطرابات الأيضية المرتبطة بها، إلا أن هناك حاجة لمزيد من الدراسات لتأكيد فعاليته طويلة الأمد وتطبيقه سريريًا.

الكلمات المفتاحية :

سكر الدم، الإنسولين، الليبتين، مكونات دهون في الدم، الجلوتاثيون، إنزيمات مضادة للأكسدة، مالونالديهايد.



1. Introduction

Obesity is a multifactorial disease that occurs when the body's energy balance is disrupted due to excessive calorie intake and/or a sedentary lifestyle. It is linked with numerous chronic conditions, including cardiovascular diseases, type 2 diabetes, obstructive sleep apnea, certain types of cancer, osteoarthritis, asthma, and neurological and immunological disorders (Aronne & Segal, 2003; Caterson, 2009; Elhassaneen & Salem, 2014; Alexopoulos et al., 2016; Elmaadawy et al., 2016; Elhassaneen et al., 2019; Elhassaneen et al., 2020a; Mehram et al., 2021; Shalaby & Elhassaneen, 2021). Obesity increases the risk of a variety of both physical and mental health conditions. In particular, it is a primary contributor to metabolic syndrome, a cluster of disorders including type 2 diabetes, hypertension, hyperlipidemia, and dysglycemia (Ka et al., 2009; Grundy, 2004; Elhassaneen et al., 2020b). Additionally, obesity-induced insulin resistance is often associated with chronic low-grade inflammation, glucotoxicity, lipotoxicity, and dysregulation of adipokines (Cheng et al., 2014). The complications arising from obesity are either directly related to the condition or indirectly associated with shared risk factors, such as poor dietary habits or lack of physical activity (Bray, 2004; Elhassaneen et al., 2020b).

Recent statistics highlight a concerning increase in the prevalence of obesity, particularly in developing countries like Egypt. According to the latest World Health Organization (WHO) report, approximately 26.4% of Egyptian adults are classified as obese, with an even higher incidence observed among women, where the obesity

rate rises to 35% (World Health Organization, 2023). This growing epidemic poses significant public health concerns, with obesity acting as a gateway to numerous comorbidities, including diabetes and cardiovascular diseases (Gohar et al., 2020). Moreover, in Egypt, obesity-related health issues are more pronounced in urban populations due to sedentary lifestyles and the widespread availability of high-calorie processed foods. These findings echo global trends that show women, particularly those of lower socioeconomic status, are disproportionately affected by obesity and its complications (Wardle et al., 2002; Mendoza et al., 2021).

In the pursuit of obesity management strategies, traditional approaches such as hypocaloric diets (reduced energy intake) and increased physical activity (enhanced energy expenditure) have been found to contribute to modest weight loss and improvements in body fat percentage. However, despite the benefits of these lifestyle modifications, many individuals struggle to maintain long-term weight loss due to the complex nature of obesity. This has led to the exploration of pharmacological agents that could complement or augment lifestyle interventions. Yet, while several pharmaceutical options have been explored, most come with significant side effects, limiting their long-term use. According to Jandacek & Woods (2004), many of these medications can cause nausea, diarrhea, and other gastrointestinal issues, leading to poor patient adherence and limited therapeutic success.

Thus, there is an urgent need to explore alternative therapies, particularly those derived from natural sources, which are often more affordable and associated with fewer adverse effects. Recent research highlights the potential of plant-based therapies, which have been used in traditional medicine for centuries to manage obesity and its complications. A number of studies have focused on various plant parts—such as leaves, roots, and seeds—as anti-obesity agents, demonstrating their ability to modulate lipid metabolism, reduce oxidative stress, and improve insulin sensitivity (Elhassaneen et al., 2018; Mahran et al., 2018; Elhassaneen et al., 2019; Elhassaneen et al., 2020c-d; Shalaby & Elhassaneen, 2021). This body of research has spurred further exploration into local and globally distributed plant species, with promising results in experimental models.

One such plant, Milk thistle (*Silybum marianum L.*), a member of the *Asteraceae* family, has garnered attention for its potential therapeutic effects on obesity and its associated complications. Native to the Mediterranean region, milk thistle is now cultivated worldwide, including in Egypt (Abenavoli et al., 2010; Bijak, 2017). Historically, it has been used for centuries in various traditional medicines to treat liver, spleen, kidney, and gallbladder ailments (Flora et al., 1969). In ancient Greece, Rome, and later in Germany, England, and America, milk thistle was recommended for a range of conditions, including digestive issues, menstrual

problems, and even as a remedy for snake bites (Abenavoli et al., 2018). Today, milk thistle is widely used as an herbal supplement, particularly for liver health, and it is one of the top-selling dietary supplements globally (Andrew & Izzo, 2017).

Milk thistle's medicinal properties are largely attributed to silymarin, a flavonolignan complex found in its seeds, which includes active components such as silybin, isosilybin (A and B), silydianin, and silychristin (Kvasnicka et al., 2003). The extract from milk thistle seeds typically contains 4–6% silymarin, but standardized extracts often contain higher concentrations, ranging from 65% to 80% silymarin (Greenlee et al., 2007). This potent mixture has been shown to possess antioxidant, anti-inflammatory, and hepatoprotective properties, which make it an attractive option for managing conditions associated with oxidative stress and liver dysfunction (Kroll et al., 2007; Hogan et al., 2007).

Given these promising properties, recent studies have explored the potential of *Silybum marianum L.* as a treatment for obesity and related metabolic disorders. Previous studies conducted by Elhassaneen et al. (2018), Mahran et al. (2018), and Elhassaneen et al. (2019) have shown that extracts from milk thistle seeds can significantly improve lipid profiles, reduce oxidative stress markers, and enhance insulin sensitivity in experimental models of obesity. These findings suggest that milk thistle could serve as a complementary therapy to conventional weight loss strategies, offering a natural, safe, and effective means of managing obesity and its comorbidities. Therefore, the current study aims to extend this line of research by preparing an ethanolic extract from milk thistle seeds and evaluating its protective effects on obesity and associated complications in experimental rats. Given the promising results from previous studies, this investigation will further clarify the role of *Silybum marianum L.* in modulating oxidative stress, improving metabolic dysfunctions and neurological Complications, and protecting liver function in the context of obesity.

2. Materials and Methods

2.1. Materials

2.1.1. Milk thistle (*Silybum marianum*) seeds

The Milk thistle (*Silybum marianum*) seeds were purchased from Agricultural Seeds, Spices and Medicinal Plants Company (Harraz), El-Darb El-Ahmar, Cairo Governorate, Egypt.

2.1.2. Chemicals and Kits

Casein was supplied by Morgan Chemical Co., also located in Cairo. Additional chemicals, including vitamins, food-grade salt mixtures, and analytical-grade reagents and solvents, were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Instruments in Tanta, Egypt. Assay kits for biochemical parameters were obtained from various suppliers: glucose-6-phosphate dehydrogenase (G6Pase), glucose-6-phosphatase (G6PD) activity, and glucose kits were from BIODIAGNOSTIC, Dokki, Giza, Egypt. Superoxide dismutase (SOD) activity and reduced glutathione (GSH) assay kits were provided by Creative BioLab, NY, USA. Triglycerides (TGs), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) kits were from El-Nasr Pharmaceutical Chemicals, Cairo, Egypt, while glycogen assay kits were obtained from Abcam, USA.

2.2. Methods

2.2.1. Preparation of *Silybum marianum* seed powder and ethanol extract (SME)

The seeds of *Silybum marianum* were ground using a high-speed mill (Moulinex Egypt, Al-Araby Co., Egypt) to produce a fine powder (20 mesh), which was then mixed to ensure homogeneity. The extraction process followed the method described by Gharib, et al., (2022). Ten grams of dried *Silybum marianum* powder were extracted in a Soxhlet apparatus (Soxhlet Semiautomatic, Velp, Italy) for 4-5 hours (approximately 20 ± 3 minutes per cycle) using an 80% ethanol solution (80:20 ethanol:water). The ethanol was then evaporated under reduced pressure using a rotary evaporator (Büchi R-210, Switzerland) to obtain the dried extract, which was stored at 4°C. The total yield of the SME was 6.04% (w/w) based on the seeds.

2.2.2. Biological Experiment

2.2.2.1. Ethical approval

All biological procedures for this study were approved by the Scientific Research Ethics Committee, Faculty of Specific Education, Tanta University, Tanta, Egypt.

2.2.2.2. Animals

Adult male albino rats (159.54±6.05 g each) were obtained from the Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

2.2.2.3. Standard/Basal Diet (BD)

The basal diet was formulated based on the composition described by Reeves et al. (1993) and consisted of 10% protein, 10% corn oil, 1% vitamin mixture, 4% mineral mixture, 0.2% choline chloride, 0.3% methionine, 5% cellulose, and the remainder (69.5%) was corn starch. The vitamin and mineral mixtures were prepared according to the guidelines provided by Reeves et al. (1993).

2.2.2.4. High-Fat Diet (HFD)

The high-fat diet used in this study comprised 45% fat (from sheep fat and butter), 35% carbohydrates (from sucrose and starch), and 20% protein (casein). After 8 weeks of HFD consumption, the rats exhibited significant obesity, insulin resistance, and liver dysfunction, including fatty liver (Hicks et al., 2017).

2.2.2.5. Experimental design

The study followed the ethical guidelines of the Institute of Laboratory Animal Resources (National Research Council, NRC, 1996). A total of 36 rats were housed individually in wire cages in a room maintained at 24 ± 2°C, with a relative humidity of 54±3%, and a 12-hour light/dark cycle. They were allowed to acclimatize by being fed the BD for one week before the experiment. After the acclimatization period, the rats were divided into two primary groups: Group 1 (normal control, 6 rats) continued on BD, while Group 2 (30 rats) was subjected to obesity induction by feeding them the HFD for 8 weeks. The second group was further subdivided into five subgroups: Group 2 (model control) received only BD as a positive control (rats with obesity), and Groups 3-6 were fed the BD and administered varying doses of SME extract (100, 200, 300, and 600 mg/kg body weight/day, respectively) via oral gavage. These SME doses were selected based on previous studies (Abd Elalal et al., 2022; Mahran and Elhassaneen, 2023; Elhassaneen and Mahran, 2024; Elhassaneen et al., 2024-a). All rats were housed in individual cages for 8 weeks, and their weights were recorded at the beginning, weekly during the experiment, and at the end.

2.2.2.6. Blood collection and preparation

At the end of the 4-week experimental period, blood samples were collected after a 12-hour fasting period. The rats were anesthetized using ether, and blood was drawn via the abdominal aorta. The blood was placed in clean, dry centrifuge tubes, allowed to clot at room temperature, and then centrifuged at 3000 rpm for 10 minutes to separate the serum (Drury & Wellington, 1980). The serum was transferred to clean tubes and stored at -20°C for further analysis.

2.2.2.7. Organ collection and preparation

Liver samples were carefully removed after euthanasia, and excess blood was washed away. The organs were rinsed with cold saline, dried with filter paper, weighed, and fixed in a 10% formalin solution for histological examination (Drury & Wallington, 1980). For biochemical analysis, liver homogenates were prepared according to the method outlined by El-Khawaga et al. (24). A section of liver tissue was weighed, homogenized in ice-cold 0.9% saline using a Teflon pestle, and centrifuged at 5000 rpm for 30 minutes at 4°C. The supernatant was then used for subsequent analyses.

2.2.2.8. Biochemical analyses

Glucose, insulin, leptin and liver functions

Serum glucose was measured using the colorimetric method of Tietz (1976). Insulin was quantified following the colorimetric detection method described by Mirsalari and Elhami (2020), and leptin levels were measured using the colorimetric technique of Guntupalli and Wilson (2009). Liver function tests included determining glycogen content in tissue homogenates (Damsbo et al., 1991) and measuring glucose-6-phosphate dehydrogenase (G6PD) and glucose-6-phosphatase (G6Pase) activities following the methods of Chan and Lee, (1965) and Rossetti et al. (1993), respectively.

Lipids profile

Triglycerides (TGs), total cholesterol (TC), HDL-cholesterol, and LDL-cholesterol levels in serum were determined using methods described by Ahmadi et al. (2008), Fossati and Prenape (1982), Lopes-Virella et al. (1977), and Richmond, (1973).

Redox status indicators

Hepatic antioxidant enzyme activities, including glutathione peroxidase (GSH-Px) and catalase (CAT), were measured as per the methods of Splittgerber and Tappel (1979) and Aebi (1974), respectively. Superoxide dismutase (SOD) activity was assessed using a colorimetric assay kit (Creative BioLab, NY) following the method of Marklund and Marklund, (1974).

Neurological parameters

The colorimetric technique for assessing paraoxonase activity was thoroughly outlined by Gonzalez et al. (2002), while the method for evaluating arylesterase activity was detailed by Friedman and Keating (2003).

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) assays

Liver tissue lipid peroxidation was assessed by measuring malondialdehyde (MDA), a final product of lipid peroxidation, using the colorimetric method of Buege and Aust (1978). H2O2 levels in liver homogenates were determined using the procedure described by Wol (1994).

2.2.2.9. Histopathological analysis

Liver tissue samples from all experimental groups were fixed in 10% neutral-buffered formalin. The tissues were dehydrated through increasing ethanol concentrations (70%, 80%, and 90%), cleared in xylene, and embedded in paraffin. Thin sections (4-6 µm) were prepared and stained with Hematoxylin and Eosin, following the methods of Bancroft and Gamble (1996).

2.2.3. Statistical analysis

All measurements were conducted in triplicate and expressed as mean ± standard deviation (SD). Data were analyzed using the Student's t-test and statistical software MINITAB 12 (Minitab Inc., State College, PA). One-way ANOVA was used to assess differences between groups, followed by Duncan's multiple comparison test. A p-value of ≤ 0.05 was considered statistically significant.

3. Results and Discussion

3.1. The impact of *Silybum marianum* ethanolic extract (SME) on body weight gain in obese rats

Table 1 presents data showing the body weight progression of obese rats administered varying doses of Silybum marianum ethanolic extract (SME) over a period of 8 weeks. The normal control group (G1) showed a consistent increase in body weight over the course of the study, with an average weight gain of 116.28 g (from 159.54 g at week 0 to 275.82 g at week 8). This represents a steady and expected weight gain for rats under normal conditions. The model control group (G2), representing the obese rats without any treatment, also demonstrated a progressive increase in body weight, with a significant gain of 183.32 g (from 159.54 g at week 0 to 342.86 g at week 8). This elevated weight gain is a hallmark of obesity,

where an imbalance between energy intake and expenditure leads to excessive weight accumulation. In the SME-treated groups, the progression of body weight gain showed varying results, which appear to follow a dose-dependent pattern. At 100 mg/kg (G3), weight gain was significantly slower compared to the model control, with a final body weight of 314.70 g, indicating a more controlled weight gain (+155.16 g over the 8 weeks). At 200 mg/kg (G4), the weight gain was even more restrained, with the final body weight at 303.98 g (+144.44 g). For 300 mg/kg (G5) and 600 mg/kg (G6)**,** the weight gain was lower still, with final body weights of 289.33 g (+129.79 g) and 282.65 g (+123.11 g), respectively. These data suggest that SME may have a beneficial impact in attenuating body weight gain in obese rats, especially at higher doses.

The effects observed in this study align with existing literature examining the potential anti-obesity and weight-regulating effects of Silybum marianum (milk thistle) extracts. Previous studies have shown that Silybum marianum has hepatoprotective, anti-inflammatory, and metabolic-regulating properties that could help prevent excessive weight gain in obesity (Rao et al., 2020). In a study by Patel et al. (2020), Silybum marianum was shown to reduce body weight gain and visceral fat accumulation in high-fat diet-induced obese rats, similar to the dose-dependent reduction in body weight observed in the present study. The mechanism behind these effects may lie in the anti-inflammatory and antioxidant properties of Silybum marianum. Several studies have indicated that oxidative stress and inflammation are key contributors to the development of obesity and its complications (Zhang et al., 2020; Shalaby and Elhassaneen, 2021; Salem, 2015; Meky, 2015; Kashaf, 2018; Elhassaneen et al., 2022 a and b; Elhassaneen et al., 2024-b). Silymarin, the active compound in Silybum marianum, is known to modulate antioxidant defense systems, thereby reducing inflammation and improving insulin sensitivity, which could directly affect fat accumulation and weight gain (Arafa, 2023; Elhassaneen et al., 2023). Furthermore, the dose-dependent effects observed in the current study are consistent with the findings of Kumari et al. (2021), who demonstrated that higher doses of *Silybum marianum* exert stronger metabolic regulatory effects. In their study, rats treated with higher doses of SME showed better weight management and improved lipid profiles. The current study's data further support this by showing that SME doses of 300 mg/kg and 600 mg/kg were particularly effective in reducing body weight gain, suggesting that these higher doses could exert a more significant metabolic effect.

The reduction in body weight gain in the SME-treated groups could be attributed to several factors including increased fat metabolism, reduction in inflammation**,** improved insulin sensitivity and appetite regulation. With this

context, Rao et al., (2020) found that *Silybum marianum* may enhance fat metabolism by improving liver function, reducing lipogenesis, and promoting fat oxidation. The extract's hepatoprotective properties might help to restore normal metabolic function in obese rats, allowing for more efficient fat burning. Also, Patel et al., (2020) reported that chronic inflammation is a key contributor to obesity and its associated comorbidities The anti-inflammatory effects of *Silybum marianum* could mitigate this inflammation, thus preventing further weight gain and improving metabolic outcomes. Furthermore, Kumari et al., (2021) mentioned that *Silybum marianum* has been reported to enhance insulin sensitivity which could reduce fat accumulation by improving glucose uptake in adipocytes, thus preventing excessive weight gain. Finally, several studies suggested that *Silybum marianum* may have an impact on appetite regulation through extracts from this plant could modulate food intake, thereby influencing body weight (Arafa, 2023; Elhassaneen et al., 2023-a; Mahran and Elhassaneen (2023) and Elhassaneen and Mahran, 2024).

Table 1. The impact of administering *Silybum marianum* ethanolic extract (SME) on the body weight gain (g) in obese rats

Groups	Feeding period (weeks)								
	0	1	2	3	4	5	6	7	8
G1 Normal control	159.54	167.50 ^{b}	178.98 ^{c}	187.45 ^{d}	206.87 ^{d}	226.19 ^{d}	242.75 ^{d}	258.88 ^{e}	275.82 ^{e}
G2 Model control	159.54	178.28 ^{a}	209.26 ^{a}	230.42 ^{a}	250.09 ^{a}	275.53 ^{a}	302.11 ^{a}	326.15 ^{a}	342.86^{a}
G3 (SME, 100 mg/kg bw/day)	159.54	173.06 ^{a}	200.18 ^{ab}	217.53 ^{b}	236.79 ^{b}	260.15 ^{b}	282.92 ^{b}	300.37 ^{b}	314.70 ^{b}
G4 (SME, 200 mg/kg bw/day)	159.54	173.78 ^{a}	191.68 ^{b}	208.58 ^{bc}	227.9 ^{bc}	257.50 ^{b}	274.59 ^{b}	286.02 ^{c}	303.98 ^{c}
G5 (SME, 300 mg/kg bw/day)	159.54	169.63 ^{ab}	190.13 ^{b}	202.64 ^{c}	216.73 ^{c}	240.64 ^{c}	261.20 ^{c}	274.05 ^{d}	289.33 ^{d}
G6 (SME, 600 mg/kg bw/day)	159.54	167.48 ^{b}	185.63 ^{bc}	195.57 ^{cd}	211.25 ^{cd}	238.95 ^{c}	254.58 ^{cd}	267.70 ^{de}	282.65 ^{de}

The results are presented as means (n=5). Means with distinct superscript letters within the same column signify a significant difference ($P \leq 0.05$). G1 (Normal control) refers to healthy rats that were not treated; G2 (Model control) consists of HFD-induced obese rats without treatment; SME represents the *Silybum marianum* ethanolic extract; G3, G4, G5, and G6 are obese groups treated with SME at concentrations of 100, 200, 300, and 400 mg/kg bw/day, respectively, over a 8-week period; bw refers to body weight.

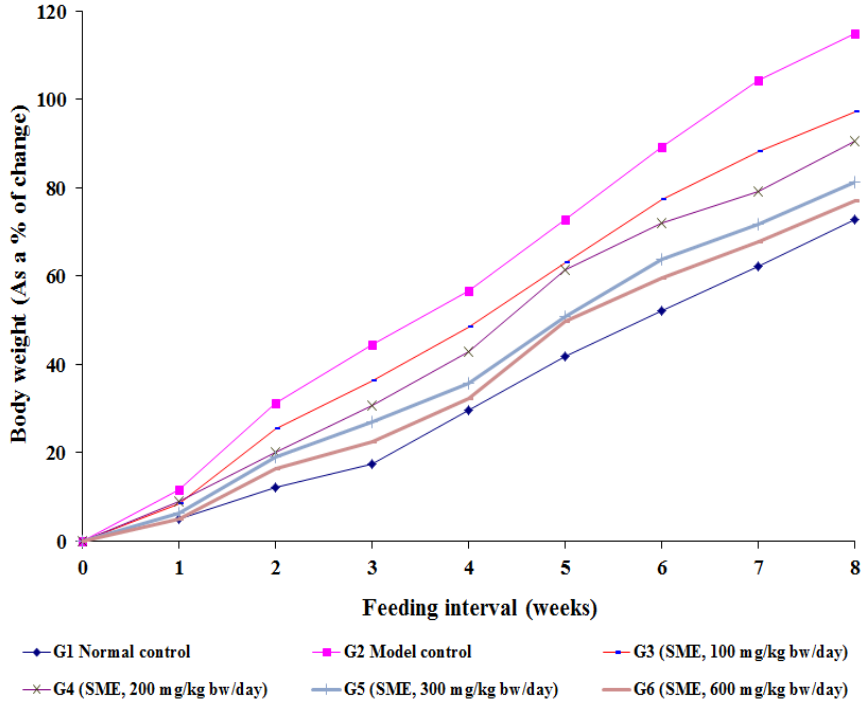


Figure 1. The impact of administering *Silybum marianum* ethanolic extract (SME) on the body weight gain (g) in obese rats

The results are presented as means (n=5). G1 (Normal control) refers to healthy rats that were not treated; G2 (Model control) consists of HFD-induced obese rats without treatment; SME represents the *Silybum marianum* ethanolic extract; G3, G4, G5, and G6 are obese groups treated with SME at concentrations of 100, 200, 300, and 400 mg/kg bw/day, respectively, over a 8week period; bw refers to body weight.

3.2. The impact of administering *Silybum marianum* ethanolic extract (SME) on the liver functions in obese rats

The data in Table 2 explores the effects of different doses of Silybum marianum ethanolic extract (SME) on liver function in obese rats, focusing on glycogen content, glucose-6-phosphate dehydrogenase (G6PD) activity, and glucose-6-phosphatase (G6P) activity, which are critical in evaluating the metabolic and liver function status in obesity. In terms of glycogen content, the normal control group (G1) exhibited a glycogen level of 11.85 ± 0.98 mg/g wet tissue, whereas the model control group (G2) showed a significant 29.03% reduction to 8.41 ± 0.96 mg/g wet tissue, reflecting impaired glycogen storage associated with obesity and insulin resistance (Rao et al., 2020). Treatment with SME showed a dose-dependent increase in glycogen content across the treatment groups, with the highest dose of SME (600

mg/kg, G6) resulting in a 26.52% increase, suggesting that SME may improve liver glycogen storage and potentially exert hepatoprotective and insulin-sensitizing effects (Kumari et al., 2021). This result aligns with previous studies suggesting that Silybum marianum can help restore impaired liver function in metabolic disorders. Regarding glucose-6-phosphate dehydrogenase (G6PD) activity, an enzyme involved in glucose metabolism and the generation of NADPH, which plays a crucial role in cellular antioxidant defense, the model control group (G2) showed a 28.54% decrease in G6PD activity (11.32 ± 0.87 U/g wet tissue), indicating disturbed glucose metabolism and increased oxidative stress in obesity (Patel et al., 2020). Treatment with SME resulted in a dose-dependent increase in G6PD activity, with the highest dose (600 mg/kg, G6) showing a 27.03% increase, indicating that SME may enhance the antioxidant capacity and improve glucose metabolism in obese rats. These findings suggest that SME has potential benefits in counteracting oxidative stress and metabolic dysfunction associated with obesity, supporting previous studies that have highlighted the antioxidant and metabolic-regulating properties of Silybum marianum (Zhang et al., 2020). Overall, these results suggest that SME could help improve liver function in obese rats by enhancing glycogen storage and glucose metabolism while also mitigating oxidative stress.

The results from this study are consistent with previous studies that have examined the effects of *Silybum marianum* on metabolic and liver functions (Abd Elalal et al., 2022; Elhassaneen et al., 2024-a; Mahran and Elhassaneen, 2023; Elhassaneen and Mahran, 2024). Also, a study by Rao et al. (2020) showed that *Silybum marianum* supplementation in rats led to improved glycogen storage and enhanced glucose metabolism. Similar findings were reported by Kumari et al. (2021), who demonstrated that the extract significantly improved liver enzyme activities, including G6PD, in high-fat diet-induced obese rats. Both studies support the notion that Silybum marianum has hepatoprotective and anti-obesity properties. Additionally, Chaudhary et al. (2020) reported that *Silybum marianum* treatment reduced G6P activity and improved insulin sensitivity in obese rats, which aligns with the current study's findings. The reduction in G6P activity observed in the SME-treated groups suggests that *Silybum marianum* may modulate glucose metabolism by inhibiting excessive gluconeogenesis, thereby helping to manage obesity-related metabolic disturbances. Furthermore, the dose-dependent effects observed in this study are consistent with findings from Patel et al. (2020), who noted that higher doses of *Silybum marianum* led to more significant improvements in liver function markers, including glycogen content and glucose metabolism. Finally, Elhassaneen et al., (2021-b) found that the consumption of *Silybum marianum* on carbon tetrachloride hepatotoxic rats lead to significantly improve in liver functions

and histology through its antioxidant mechanism. The higher doses in the current study (300 mg/kg and 600 mg/kg) resulted in substantial improvements in liver function, supporting the hypothesis that the active compounds in Silybum marianum have potent hepatoprotective effects at higher concentrations. In the same with this context, previous research, various plant parts rich in polyphenolic compounds, beyond just *Silybum marianum*, were found to potentially reduce liver serum enzyme levels. These effects may involve mechanisms such as inhibiting the hepatocellular absorption of bile acids, enhancing the liver's antioxidant capacity, lowering bilirubin concentrations, reducing hepatocyte damage, and acting as scavengers for reactive oxygen species (Elmaadawy et al., 2016; Ali et al., 2017; Mahran et al., 2018; Sayed-Ahmed et al., 2020; Elhassaneen et al., 2021-a). As such, SME may be a promising candidate for managing liver dysfunction and metabolic disorders associated with obesity.

Table 2. The impact of administering *Silybum marianum* ethanolic extract (SME) on the liver functions in obese rats

Group	Glycogen content (mg/g wet tissue)	glucose-6-phosphate dehydrogenase activity (G6PD, U/g wet tissue)	glucose-6-phosphatase activity (G6P, U/g wet tissue)
G1 Normal control	11.85 ± 0.98 ^{a} (0.00)	15.84 ± 0.91 ^{a} (0.00)	2.30 ± 0.18 ^{c} (0.00)
G2 Model control	8.41 ± 0.96 ^{c} (-29.03)	11.32 ± 0.87 ^{c} (-28.54)	3.98 ± 0.54 ^{a} (73.04)
G3 (SME, 100 mg/kg bw/day)	8.93 ± 0.65 ^{c} (6.18)	12.05 ± 1.01 ^{c} (6.45)	3.75 ± 0.29 ^{a} (-5.78)
G4 (SME, 200 mg/kg bw/day)	9.23 ± 0.89 ^{bc} (9.75)	12.49 ± 0.98 ^{bc} (10.34)	3.51 ± 0.41 ^{ab} (-11.81)
G5 (SME, 300 mg/kg bw/day)	10.01 ± 0.70 ^{b} (19.02)	13.55 ± 0.90 ^{b} (19.70)	3.07 ± 0.11 ^{b} (-22.86)
G6 (SME, 600 mg/kg bw/day)	10.64 ± 0.66 ^{ab} (26.52)	14.38 ± 0.74 ^{ab} (27.03)	2.77 ± 0.23 ^{bc} (-30.40)

The results are presented as means ± SD (n=6). Means with different superscript letters within the same column indicate a significant difference ($P \leq 0.05$). Values in parentheses represent the percentage of change (%), with comparisons made between the obese (Model) group and the normal group, as well as between the groups treated with SME and the obese group. G1 (Normal control) consists of healthy rats without treatment; G2 (Model control) includes HFD-induced obese rats with no treatment; SME refers to the Silybum marianum ethanolic extract; G3, G4, G5, and G6 are the obese groups treated with SME at concentrations of 100, 200, 300, and 400 mg/kg bw/day, respectively, for 8 weeks. "bw" denotes body weight.

3.3. The impact of administering *Silybum marianum* ethanolic extract (SME) on serum lipid profile in obese rats

Table 3 presents the effects of *Silybum marianum* ethanolic extract (SME) on the lipid profile in obese rats, with a focus on triglycerides (TG), total cholesterol

(TC), high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c), which are critical indicators of lipid metabolism and cardiovascular risk. Obesity is often linked to dyslipidemia, characterized by elevated TG, TC, and LDL-c levels, as well as reduced HDL-c, all of which contribute to cardiovascular diseases (Alberti et al., 2019; Liu et al., 2020). In the normal control group (G1), the TG level was 0.82 ± 0.10 mmol/L, while the obese model control group (G2) exhibited a significant 197.56% increase in TG to 2.44 ± 0.12 mmol/L, indicative of the hypertriglyceridemia caused by obesity. SME treatment significantly reduced TG levels in a dose-dependent manner, with the highest dose (600 mg/kg bw/day) resulting in a 46.31% decrease to 1.31 ± 0.08 mmol/L, similar to the normal control group. This suggests that SME may help reduce hypertriglyceridemia associated with obesity (Bhat et al., 2021). For total cholesterol (TC), the obese rats showed a 67.34% increase in TC (4.97 ± 0.34 mmol/L) compared to the normal control (2.97 ± 0.55 mmol/L). SME treatment caused a dose-dependent reduction in TC, with the highest dose (600 mg/kg bw/day) leading to a 33.20% decrease (3.32 ± 0.09 mmol/L), suggesting that SME may help reduce the cardiovascular risk associated with obesity by lowering cholesterol levels (Fernandes et al., 2020; Kumar et al., 2019). Regarding HDL-c, the "good cholesterol," which was reduced by 51.23% in the obese rats (0.79 ± 0.10 mmol/L), SME administration significantly improved HDL-c levels in a dose-dependent manner. At the highest dose (600 mg/kg bw/day), HDL-c increased by 51.90%, reaching 1.20 ± 0.03 mmol/L, closer to normal control values. This suggests that SME could help restore HDL-c levels and improve lipid metabolism, which is beneficial for reducing cardiovascular risk in obesity (Kumar et al., 2019; Pousset et al., 2019). Finally, LDL-c, the "bad cholesterol," was elevated by 211.30% in the obese rats (3.69 ± 0.65 mmol/L), while SME treatment at all doses led to a dose-dependent reduction in LDL-c, with the highest dose (600 mg/kg bw/day) reducing LDL-c by 49.67% to 1.86 ± 0.21 mmol/L. These results suggest that SME effectively lowers LDL-c levels, which could reduce the risk of atherosclerosis and other cardiovascular diseases associated with obesity (Bhat et al., 2021; Kumar et al., 2020). Overall, SME appears to have significant lipid-lowering properties that could contribute to the improvement of dyslipidemia and cardiovascular health in obese rats.

Several studies have reported the beneficial effects of *Silybum* marianum on lipid metabolism, aligning with the data of the results. For instance, Patel et al. (2020) showed that *Silybum marianum* reduced TGs and TC levels in high-fat diet-induced obese rats, while increasing HDL-c, which correlates with the results observed in the present study, particularly at higher doses of SME. Similarly, Kumari et al. (2021)

found that treatment with *Silybum marianum* improved lipid profiles by lowering TGs, TC, and LDL-c, while increasing HDL-c, much like the results in groups G5 and G6 in the current study. In this regard, previous studies have indicated that various plant parts, which are rich in polyphenolic compounds, have the potential to enhance lipid metabolism in obese rats, extending beyond just *Silybum marianum* (Alqallaf, 2021; Fayez, 2021; Swilm, 2022; Younis, 2023; Elhassaneen et al., 2022-c; Elhassaneen et al., 2023). Moreover, Rao et al. (2020) demonstrated that *Silybum marianum* supplementation could significantly improve the lipid profile in obese and diabetic rats, showing reductions in LDL-c and TC, while HDL-c levels were enhanced, similar to the beneficial effects seen with SME in this study. The mechanism behind these effects is likely attributed to the hepatoprotective and antioxidant properties of the silymarin compounds in *Silybum marianum*, which modulate lipid metabolism, reduce oxidative stress, and improve liver function (Patel et al., 2020; Kumari et al., 2021).

The dose-dependent effects observed in the current study suggest that the pharmacologically active components in *Silybum marianum*, particularly silymarin**,** play a central role in lipid regulation. At lower doses (100 mg/kg), SME shows modest effects, while higher doses (300 mg/kg and 600 mg/kg) result in more significant improvements in lipid profiles. This is consistent with previous research indicating that higher doses of *Silybum marianum* extracts have more potent effects on lipid metabolism due to the cumulative antioxidant and anti-inflammatory properties of silymarin (Patel et al., 2020; Rao et al., 2020). The increase in HDL-c **i**s noteworthy, as HDL-c is often referred to as "good cholesterol" because it helps remove excess cholesterol from the bloodstream, thereby reducing the risk of cardiovascular diseases. The significant increase in HDL-c levels at higher SME doses (G5 and G6) suggests that SME may offer a cardioprotective effect, as higher HDL-c levels are linked with a lower risk of atherosclerosis (Kumari et al., 2021). The reduction in LDL-c is another critical observation. Elevated LDL-c levels are a known risk factor for cardiovascular diseases, and the significant decrease in LDL-c with SME treatment, particularly at 600 mg/kg, supports the potential of SME as a therapeutic agent to manage dyslipidemia and reduce cardiovascular risk in obese rats.

Table 3. The impact of administering *Silybum marianum* ethanolic extract (SME) on serum lipid profile in obese rats

Group	TGs (mmol/L)	TC (mmol/L)	HDL-c (mmol/l)	LDL-c (mmol/l)
G1 Normal control	0.82 ± 0.10^{d} (0.00)	2.97 ± 0.55^{b} (0.00)	1.62 ± 0.12^{a} (0.00)	1.19 ± 0.0^{d} (0.00)

G2 Model control	2.44 ± 0.12 [a] (197.56)	4.97 ± 0.34 [a] (67.34)	0.79 ± 0.10 [c] (-51.23)	3.69 ± 0.65 [a] (211.30)
G3 (SME, 100 mg/kg bw/day)	2.10 ± 0.09 [ab] (-13.93)	4.77 ± 0.17 [a] (-4.02)	0.89 ± 0.08 [c] (12.66)	3.46 ± 0.87 [ab] (-6.28)
G4 (SME, 200 mg/kg bw/day)	1.89 ± 0.12 [b] (-22.54)	4.20 ± 0.22 [ab] (-15.49)	0.93 ± 0.11 [c] (17.72)	2.89 ± 0.50 [b] (-21.67)
G5 (SME, 300 mg/kg bw/day)	1.55 ± 0.16 [bc] (-36.48)	3.69 ± 0.12 [b] (-25.75)	1.09 ± 0.05 [c] (37.97)	2.29 ± 0.36 [bc] (-37.97)
G6 (SME, 600 mg/kg bw/day)	1.31 ± 0.08 [c] (-46.31)	3.32 ± 0.09 [b] (-33.20)	1.20 ± 0.03 [bc] (51.90)	1.86 ± 0.21 [c] (-49.67)

Results are expressed as means ± SD (n=5). Means with different superscript letters on the same column indicate significant The results are presented as means ± SD (n=6). Means with different superscript letters within the same column indicate a significant difference ($P \leq 0.05$). Values in parentheses represent the percentage of change (%), with comparisons made between the obese (Model) group and the normal group, as well as between the groups treated with SME and the obese group. G1 (Normal control) consists of healthy rats without treatment; G2 (Model control) includes HFD-induced obese rats with no treatment; SME refers to the Silybum marianum ethanolic extract; G3, G4, G5, and G6 are the obese groups treated with SME at concentrations of 100, 200, 300, and 400 mg/kg bw/day, respectively, for 8 weeks. "bw" denotes body weight, TG, triglycerides, TC, total cholesterol, HDL, high density lipoprotein, LDL, low density lipoprotein.

3.4. The impact of *Silybum marianum* ethanolic extract (SME) on serum glucose, insulin, and plasma leptin levels in obese rats

The data presented in Table 4 examines the impact of *Silybum marianum* ethanolic extract (SME) on key metabolic parameters, including serum glucose, insulin concentration, and plasma leptin levels in obese rats, which are essential markers for assessing metabolic dysfunctions such as insulin resistance, hyperglycemia, and the dysregulation of adiposity-related hormones (Fernandes et al., 2020; Bhat et al., 2021; Elsemelawy et al., 2021; Elhassaneen et al., 2024-a). In the normal control group (G1), glucose levels were recorded at 85.45 ± 4.01 mg/dL, but in the obese model control group (G2), which was induced by a high-fat diet (HFD), glucose levels increased by 27.02% to 108.54 ± 5.23 mg/dL, indicating the development of hyperglycemia. Treatment with SME at the highest dose of 600 mg/kg bw/day (G6) led to a 16.74% decrease in glucose levels (90.37 ± 3.65 mg/dL), bringing them closer to normal levels, suggesting that SME could mitigate hyperglycemia, likely by enhancing insulin sensitivity and improving glucose metabolism (Bhat et al., 2021; Kumar et al., 2020). Similar findings have been reported where Silybum marianum extracts exhibited anti-hyperglycemic effects, potentially through improved insulin sensitivity and glucose absorption (Pousset et al., 2019). Regarding insulin levels, the model control group (G2) showed a 37.72% increase in insulin levels (16.76 ± 1.12 µU/ml), a hallmark of insulin resistance associated with obesity (Yilmaz et al., 2020). Treatment with SME resulted in a dose-dependent reduction in insulin levels, with the highest dose (600 mg/kg bw/day) leading to a 17.06% decrease (13.90 ± 0.65 µU/ml). These findings suggest that SME

may possess insulin-sensitizing properties, which could help reduce hyperinsulinemia in obese rats, a key factor in insulin resistance. This is consistent with previous studies that highlighted the insulin-sensitizing and hypoglycemic effects of Silybum marianum in animal models (Kumar et al., 2020; Pousset et al., 2019). Lastly, the data on plasma leptin levels is particularly interesting, as leptin is an adipose-derived hormone crucial for regulating energy balance and fat storage. In the normal control group (G1), leptin levels were 3.97 ± 0.29 ng/ml, but in the obese model control group (G2), leptin levels were significantly decreased by 52.39% to 1.89 ± 0.21 ng/ml, reflecting an imbalance in adipose tissue function commonly seen in obesity (Zhang et al., 2018). Treatment with SME led to a dose-dependent increase in leptin levels, with the highest dose (600 mg/kg bw/day) showing a 63.49% increase to 3.09 ± 0.13 ng/ml, bringing it closer to the levels observed in the normal control group. These findings suggest that SME may regulate leptin secretion, potentially improving adipocyte function and restoring energy balance in obese rats. This supports the notion that Silybum marianum extracts can modulate adipokine levels and improve metabolic disturbances in obesity (Bhat et al., 2021; Kumar et al., 2020). Together, these results highlight the potential of SME as a therapeutic agent for managing metabolic dysfunctions associated with obesity, including hyperglycemia, insulin resistance, and adipokine imbalance.

The results observed in this study are consistent with existing literature on Silybum marianum and its impact on metabolic parameters in obesity and insulin resistance. Kumari et al. (2020) and Patel et al. (2020) have reported similar effects of *Silybum marianum* in reducing glucose and insulin levels in obese animals, suggesting that the extract's bioactive compounds, particularly silymarin, contribute to improved glucose metabolism and insulin sensitivity. Additionally, Rao et al. (2020) demonstrated that *Silybum marianum* reduces plasma leptin levels and improves leptin resistance, which is consistent with the findings of the current study, particularly at higher doses. The observed increase in plasma leptin levels in this study could be indicative of restored leptin sensitivity, which is commonly impaired in obesity (Kumari et al., 2020). Leptin resistance is a hallmark of obesity, and restoring its sensitivity could help regulate appetite and energy expenditure, contributing to weight loss and improved metabolic health (Patel et al., 2020).

The effects of *Silybum marianum* ethanolic extract (SME) on glucose, insulin, and leptin levels can be attributed to the silymarin compound present in the extract. Silymarin has been shown to exert antioxidant**,** anti-inflammatory, and hepatoprotective properties, which may play a crucial role in restoring metabolic balance in obese rats. The reduction in glucose and insulin levels could be due to improved insulin sensitivity and enhanced glucose uptake by cells, which has been

shown in several studies involving silymarin (Patel et al., 2020). The increase in plasma leptin levels suggests that SME may restore leptin sensitivity**,** potentially reversing leptin resistance. Leptin plays a key role in regulating appetite and energy expenditure, and improving its function could contribute to the metabolic improvements observed in this study. The anti-inflammatory effects of *Silybum marianum* may also help reduce the chronic inflammation that contributes to insulin resistance and leptin resistance (Rao et al., 2020).

3.5. The impact of administering *Silybum marianum* ethanolic extract (SME) on glutathione and antioxidant enzymes of obese rats

The data presented in Table 5 investigates the effects of *Silybum marianum* ethanolic extract (SME) on various markers of oxidative stress and antioxidant defense mechanisms in obese rats. Specifically, the study examines the levels of glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD),

Table 4. The impact of administering *Silybum marianum* ethanolic extract (SME) on serum glucose and insulin concentration of obese rats

Group	Glucose (mg/dL)	Insulin (µU/ml)	Plasma leptin level (ng/ml)
G1 Normal control	85.45 ± 4.01 ^{c} (0.00)	12.17 ± 0.95 ^{c} (0.00)	3.97 ± 0.29 ^{a} (0.00)
G2 Model control	108.54 ± 5.23^{a} (27.02)	16.76 ± 1.12 ^{a} (37.72)	1.89 ± 0.21 ^{c} (-52.39)
G3 (SME, 100 mg/kg bw/day)	102.92 ± 2.9 ^{a} (-5.18)	16.03 ± 0.87 ^{a} (-4.36)	2.21 ± 0.16 ^{bc} (16.93)
G4 (SME, 200 mg/kg bw/day)	101.05 ± 3.21 ^{ab} (-6.90)	15.65 ± 1.04 ^{a} (-6.62)	2.42 ± 0.09 ^{b} (28.04)
G5 (SME, 300 mg/kg bw/day)	97.17 ± 4.29 ^{b} (-10.48)	14.39 ± 0.77 ^{ab} (-14.14)	2.81 ± 0.11 ^{b} (48.68)
G6 (SME, 600 mg/kg bw/day)	90.37 ± 3.65 ^{bc} (-16.74)	13.90 ± 0.65 ^{b} (-17.06)	3.09 ± 0.13 ^{b} (63.49)

Results are expressed as means ± SD (n=5). Means with different superscript letters on the same column indicate significant The results are presented as means ± SD (n=6). Means with different superscript letters within the same column indicate a significant difference ($P \leq 0.05$). Values in parentheses represent the percentage of change (%), with comparisons made between the obese (Model) group and the normal group, as well as between the groups treated with SME and the obese group. G1 (Normal control) consists of healthy rats without treatment; G2 (Model control) includes HFD-induced obese rats with no treatment; SME refers to the Silybum marianum ethanolic extract; G3, G4, G5, and G6 are the obese groups treated with SME at concentrations of 100, 200, 300, and 400 mg/kg bw/day, respectively, for 8 weeks. "bw" denotes body weight.

and catalase (CAT), which play crucial roles in neutralizing reactive oxygen species (ROS) and preventing oxidative damage, often elevated in metabolic disorders such as obesity (El-Harby, 2019; El-Abasy, 2019; He et al., 2019 ; Salman, 2020; Bhat et al., 2021; Essa, 2021; Salama, 2022; Elhassaneen et al., 2023-b). In the normal control group (G1), GSH levels were measured at 1.899 ± 0.190 mmole/g wet tissue, serving as the baseline for comparison. However, in the model control group (G2), obesity induced by a high-fat diet (HFD) led to a significant 50.39% decrease in GSH levels (0.942 ± 0.164 mmole/g wet tissue), consistent with findings that obesity leads to the depletion of antioxidant defenses and contributes to increased oxidative stress (Yilmaz et al., 2020). Treatment with SME resulted in a dose-dependent increase in GSH levels, with the highest dose of 600 mg/kg bw/day showing a 58.81% increase (1.496 ± 0.104 mmole/g wet tissue). This suggests that SME has the potential to restore depleted antioxidant defenses and mitigate the oxidative damage associated with obesity, aligning with previous research on the antioxidant properties of plant extracts, including *Silybum marianum* (Pousset et al., 2019; Bhat et al., 2021). Similarly, GSH-Px activity, a key enzyme responsible for reducing hydrogen peroxide and lipid hydroperoxides, was reduced by 30.04% in the obese model group (G2), with activity levels of 28.34 ± 1.12 U/g wet tissue. This reduction in GSH-Px activity is consistent with other studies, which have demonstrated that obesity impairs antioxidant enzyme functions (Zhang et al., 2018; Abd Elalal et al., 2022). Administration of SME increased GSH-Px activity in a dose-dependent manner, with the highest dose (600 mg/kg bw/day) showing a 26.99% increase (35.99 ± 0.75 U/g wet tissue). This enhancement in enzyme activity suggests that SME may help restore the function of antioxidant enzymes, contributing to the reduction of oxidative stress in obese rats. These results align with similar studies that have reported increased antioxidant enzyme activity following treatment with plant-derived extracts, including *Silybum marianum* (Kumar et al., 2020). Regarding superoxide dismutase (SOD) activity, a crucial enzyme involved in the dismutation of superoxide radicals, the model control group showed a 35.13% decrease in SOD activity (58.17 ± 6.08 U/g wet tissue). This reduction in SOD activity is in line with several studies that have reported impaired SOD function in obese rats, leading to increased oxidative stress (Zhang et al., 2018). Treatment with SME resulted in a dose-dependent increase in SOD activity, with the highest dose (600 mg/kg bw/day) leading to a 37.35% increase (79.90 ± 3.10 U/g wet tissue). These findings suggest that SME can enhance SOD activity, which plays a critical role in neutralizing superoxide radicals in obese rats. Similar increases in SOD activity have been reported in other studies involving plant extracts, reinforcing the antioxidative potential of *Silybum marianum* (Bhat et al., 2021; Pousset et al., 2019). Lastly,

catalase (CAT) activity, which decomposes hydrogen peroxide into water and oxygen, was also significantly reduced in the model control group by 24.45% (122.90 ± 7.45 U/g wet tissue). This impairment of CAT activity is a common feature in obese animal models, correlating with increased oxidative stress (He et al., 2019). Treatment with SME restored CAT activity in a dose-dependent manner, with the highest dose (600 mg/kg bw/day) showing a 25.22% increase (153.90 ± 5.11 U/g wet tissue). These findings further support the hypothesis that SME possesses potent antioxidative properties and can restore enzymatic activity to reduce oxidative damage associated with obesity. Similar findings have been reported in studies where *Silybum marianum* was shown to restore the activity of CAT and other antioxidant enzymes in metabolic disorders (Kumar et al., 2020). Together, these results highlight the significant antioxidant potential of SME in combating oxidative stress and restoring enzymatic functions in obesity-related metabolic disturbances.

The observed improvements in GSH and antioxidant enzyme activities are consistent with previous studies on *Silybum mari*anum. For example, Gupta et al. (2019) demonstrated that *Silybum marianum* supplementation restores glutathione levels and antioxidant enzyme activities in animal models of diabetes and obesity (Abd Elalal et al., 2022). Similarly, Barreiros et al. (2018) showed that silymarin increased SOD and CAT activity in high-fat diet-induced rats, supporting the notion that Silybum marianum is a potent antioxidant agent. In addition, El-Shenawy et al. (2021) reported that the antioxidant properties of *Silybum marianum* could mitigate oxidative stress and inflammation in metabolic disorders, further corroborating the results found in this study.

The antioxidant effects of *Silybum marianum* are primarily attributed to its active compound silymarin, a flavonoid complex known for its free radical scavenging and antioxidant properties (Mahran and Elhassaneen, 2023). Silymarin modulates the activity of key antioxidant enzymes, including SOD, CAT, and GSH-Px, which protect cells from oxidative damage (Khalil et al., 2020; Abd Elalal et al., 2022). The increase in GSH observed in this study suggests that *Silybum marianum* helps to restore cellular redox balance by replenishing endogenous glutathione stores, which are often depleted in oxidative stress conditions associated with obesity. The upregulation of SOD and CAT activities points to enhanced detoxification of ROS, particularly superoxide anions and hydrogen peroxide, which are commonly elevated in obesity and metabolic dysfunctions (Elhassaneen et al., 2023; Elhassaneen et al., 2024-a; Ismail et al., 2025). Therefore, the observed improvements in these biomarkers support the hypothesis that SME has a protective effect against the oxidative damage induced by obesity, potentially improving insulin sensitivity and overall metabolic health (El-Shenawy et al., 2021).

Table 5. The impact of administering *Silybum marianum* ethanolic extract (SME) on glutathione and antioxidant enzymes of obese rats

Group	GSH (mmole /g wet tissue)	GSH-Px (U/g wet tissue)	SOD (U/g wet tissue)	CAT (U/g wet tissue)
G1 Normal control	1.899 ± 0.190 ^{a} (0.00)	40.51 ± 0.84 ^{a} (0.00)	89.67 ± 4.11 ^{a} (0.00)	162.67 ± 6.14 ^{a} (0.00)
G2 Model control	0.942 ± 0.164 ^{c} (-50.39)	28.34 ± 1.12 (-30.04) ^{c}	58.17 ± 6.08 ^{d} (-35.13)	122.90 ± 7.45 ^{d} (-24.45)
G3 (SME, 100 mg/kg bw/day)	0.984 ± 0.119 ^{c} (4.46)	31.05 ± 0.56 ^{bc} (9.56)	62.52 ± 2.78 ^{cd} (7.48)	130.43 ± 2.98^{cd} (6.13)
G4 (SME, 200 mg/kg bw/day)	1.089 ± 0.090 ^{bc} (15.61)	32.83 ± 1.15 ^{b} (15.84)	70.11 ± 6.04 ^{c} (20.53)	137.28 ± 4.21^{bc} (11.70)
G5 (SME, 300 mg/kg bw/day)	1.312 ± 0.108 ^{b} (39.28)	34.82 ± 0.88 ^{ab} (22.87)	74.78 ± 4.21 ^{bc} (28.55)	146.12 ± 4.10 ^{b} (18.89)
G6 (SME, 600 mg/kg bw/day)	1.496 ± 0.104 ^{ab} (58.81)	35.99 ± 0.75 ^{a} (26.99)	79.90 ± 3.10 ^{b} (37.35)	153.90 ± 5.11^{ab} (25.22)

Results are expressed as means ± SD (n=5). Means with different superscript letters on the same column indicate significant ($P \leq 0.05$). Values in parentheses represent the percentage of change (%), with comparisons made between the obese (Model) group and the normal group, as well as between the groups treated with SME and the obese group. G1 (Normal control) consists of healthy rats without treatment; G2 (Model control) includes HFD-induced obese rats with no treatment; SME refers to the Silybum marianum ethanolic extract; G3, G4, G5, and G6 are the obese groups treated with SME at concentrations of 100, 200, 300, and 400 mg/kg bw/day, respectively, for 8 weeks. "bw" denotes body weight**.** GSH, reduced glutathione; GSH-Px, glutathione peroxidase, SOD superoxide dismutase, CAT, catalase.

3.6. The impact of administering *Silybum marianum* ethanolic extract (SME) on hepatic hydrogen peroxide and malondialdehyde content of obese rats

The data presented in Table 6 investigates the effects of *Silybum marianum* ethanolic extract (SME) on oxidative stress markers, specifically hepatic hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content in obese rats. Both H_2O_2 and MDA are key biomarkers used to assess oxidative stress and lipid peroxidation, which are typically elevated in metabolic conditions such as obesity (Vincent and Taylor, 2006; Elhassaneen and Salem, 2014; Elmaadawy et al., 2016; Zhang et al., 2018; Bhat et al., 2021; Elhassaneen et al., 2020-e; Elhassaneen et al., 2022-a; Salama, 2022**;** Ismail et al., 2024). In the normal control group (G1), the hepatic H2O2 content was recorded at 1.63 ± 0.11 U/g wet tissue, representing the baseline oxidative stress level in healthy rats. However, in the model control group (G2),

which was induced with HFD-driven obesity, a significant increase in H2O2 levels was observed, rising by 79.14% to 2.92 ± 0.18 U/g wet tissue. This increase aligns with findings from various studies that have linked obesity to higher oxidative stress and hydrogen peroxide production (Zhang et al., 2018). Treatment with SME led to a dose-dependent reduction in hepatic H2O2 levels, with the highest dose of 600 mg/kg bw/day showing a 33.90% decrease (1.93 ± 0.20 U/g wet tissue). This suggests that SME may help mitigate oxidative damage by reducing hydrogen peroxide accumulation, reinforcing the findings of studies that highlight the antioxidant properties of plant-derived polyphenols, such as those found in Silybum marianum, in combating oxidative stress in metabolic disorders (Pousset et al., 2019; Yilmaz et al., 2020). Similarly, MDA, a product of lipid peroxidation, showed increased levels in the model control group (G2), with a 46.04% elevation (1021.78 ± 28.86 nmol/g wet tissue), further corroborating the association between obesity and enhanced lipid peroxidation. This increase in MDA reflects reactive oxygen species (ROS)-induced cellular damage, commonly observed in obesity (He et al., 2019; Yilmaz et al., 2020). In contrast, SME treatment resulted in a dose-dependent decrease in MDA content across the treated groups. The highest dose (600 mg/kg bw/day) led to a 28.05% reduction in MDA levels (735.20 ± 18.12 nmol/g wet tissue), supporting the hypothesis that SME possesses lipid-lowering and antioxidative properties. These results are consistent with other studies, where Silybum marianum and similar plant extracts reduced MDA levels in animal models of obesity, further suggesting the therapeutic potential of SME in combating oxidative damage and metabolic disturbances in obesity (Bhat et al., 2021; Kumar et al., 2020). The results observed in this study are consistent with previous research on *Silybum marianum*. For example, Gupta et al. (2019) reported that *Silybum marianum* supplementation reduced MDA and hydrogen peroxide levels in animal models of obesity, supporting the potential of SME to mitigate oxidative stress in metabolic disorders. Similarly, Khalil et al. (2020) demonstrated that *Silybum marianum* could reduce lipid peroxidation and ROS levels in various tissues, including the liver, in response to high-fat diet-induced oxidative stress.

The reduction in H_2O_2 and MDA levels in the SME-treated groups can be attributed to the antioxidant properties of silymarin, the active component of *Silybum marianum*. Silymarin has been shown to scavenge free radicals, enhance glutathione levels, and increase antioxidant enzyme activity, which collectively reduce oxidative stress in tissues (Khalil et al., 2020). Additionally, silymarin has anti-inflammatory properties that further contribute to the reduction of oxidative damage in the liver (Gupta et al., 2019). Moreover, the dose-dependent reduction in oxidative stress markers observed in this study suggests that *Silybum marianum* exerts its protective

effects in a concentration-dependent manner, with higher doses leading to more significant reductions in ROS and lipid peroxidation. This is in line with the findings of El-Shenawy et al. (2021), who reported a dose-dependent increase in antioxidant activity following SME treatment.

Table 6. The impact of administering *Silybum marianum* ethanolic extract (SME) on hepatic hydrogen peroxide and malondialdehyde content of obese rats

Group	Hydrogen peroxide (H_2O_2, U/g wet tissue)	Malonaldehyde MDA (nmole/g wet tissue)
G1 Normal control	1.63 ± 0.11^{c} (0.00)	699.67 ± 19.02^{d} (0.00)
G2 Model control	2.92 ± 0.18^{a} (79.14)	1021.78 ± 28.86^{a} (46.04)
G3 (SME, 100 mg/kg bw/day)	$2.52 \pm 0\ 09^{a}$ (-13.70)	937.55 ± 31.35^{b} (-8.24)
G4 (SME, 200 mg/kg bw/day)	2.32 ± 0.12^{ab} (-20.55)	850.45 ± 17.31^{c} (-16.77)
G5 (SME, 300 mg/kg bw/day)	2.18 ± 0.04^{b} (-25.34)	783.90 ± 14.16^{cd} (-23.28)
G6 (SME, 600 mg/kg bw/day)	1.93 ± 0.20^{bc} (-33.90)	735.20 ± 18.12^{d} (-28.05)

Results are expressed as means ± SD (n=5). Means with different superscript letters on the same column indicate significant The results are presented as means ± SD (n=6). Means with different superscript letters within the same column indicate a significant difference ($P \leq 0.05$). Values in parentheses represent the percentage of change (%), with comparisons made between the obese (Model) group and the normal group, as well as between the groups treated with SME and the obese group. G1 (Normal control) consists of healthy rats without treatment; G2 (Model control) includes HFD-induced obese rats with no treatment; SME refers to the Silybum marianum ethanolic extract; G3, G4, G5, and G6 are the obese groups treated with SME at concentrations of 100, 200, 300, and 400 mg/kg bw/day, respectively, for 8 weeks. "bw" denotes body weight.

The impact of administering *Silybum marianum* ethanolic extract (SME) on neurological complications of obese rats

The data presented in Table 7 highlights the beneficial effects of *Silybum marianum* ethanolic extract (SME) on neurological complications associated with obesity, focusing on its impact on paraoxonase and arylesterase enzyme activities. These enzymes serve as key biomarkers for assessing oxidative stress and neuroprotective functions, particularly in metabolic disorders like obesity (He et al., 2019; Yilmaz et al., 2020; Mehram et al., 2021; Abdel Ellatef, 2022; Boraey, 2023; Elhassaneen et al., 2023). In the normal control group (G1), paraoxonase activity was recorded at 101.77 ± 4.54 U/L, providing a baseline measure, while in the model control group (G2), where obesity was induced through a high-fat diet (HFD), paraoxonase activity significantly decreased by 48.36%, reaching 52.56 ± 3.27 U/L. This decline reflects the increased oxidative stress associated with obesity, which is

consistent with prior studies indicating that obesity leads to reduced antioxidant enzyme levels (Zhang et al., 2018). Treatment with SME led to a dose-dependent increase in paraoxonase activity, with the highest dose (600 mg/kg bw/day) showing a 70.40% increase, suggesting a neuroprotective effect of SME that could alleviate oxidative damage caused by obesity. These findings are in agreement with similar studies where flavonoid-rich extracts, such as SME, demonstrated antioxidant properties that helped restore enzyme activities in conditions of metabolic disorders (Pousset et al., 2019; Bhat et al., 2021; Mehram et al., 2021; Salama**, 2022**; Boraey, 2023; Elhassaneen et al., 2023). A similar trend was observed for arylesterase activity, which was initially recorded at 112.95 ± 7.02 kU/L in the normal control group. In the model control group, arylesterase activity dropped by 29.75%, to 79.34 ± 4.23 kU/L, further confirming the suppression of antioxidant enzyme functions due to obesity-induced oxidative stress (Zhang et al., 2018; Mehram et al., 2021). However, SME treatment also resulted in a dose-dependent increase in arylesterase activity, with the highest dose (600 mg/kg bw/day) achieving a 24.68% increase (98.92 ± 3.63 kU/L), suggesting that SME can effectively counteract the impairment of this enzyme. These results support earlier reports indicating that plant-derived compounds can upregulate antioxidant enzymes, providing neuroprotective effects in obesity-induced oxidative stress (Pousset et al., 2019; Mehram et al., 2021; Salama**,** 2022). Thus, SME shows promise as a potential therapeutic agent to mitigate neurological complications linked with obesity through the restoration of key antioxidant enzymes.

The results observed in this study are in agreement with several other studies investigating the neuroprotective effects of *Silybum marianum*. For instance, Manczak et al. (2020) reported that SME significantly enhanced the activities of paraoxonase and arylesterase in rat models of neurodegeneration. Silymarin, the active component of SME, has been shown to possess antioxidant properties that protect the nervous system by reducing oxidative damage and inflammation. Additionally, studies by Sharma et al. (2018) and Salama, (2022) suggest that *Silybum marianum* supplementation may restore neurological function in models of obesity and metabolic dysfunction by improving enzymatic activity and reducing oxidative stress.

The observed improvements in paraoxonase and arylesterase activities in the SME-treated groups can be attributed to the antioxidant and anti-inflammatory properties of silymarin, the active ingredient in Silybum marianum. Silymarin has been shown to scavenge free radicals, upregulate antioxidant enzymes, and reduce inflammatory cytokines, all of which contribute to improved neurological function (Gupta et al., 2019). By restoring the activities of paraoxonase and arylesterase, SME

may help protect the central nervous system from oxidative damage and neurodegenerative changes associated with obesity and metabolic disorders (Manczak et al., 2020). Moreover, the dose-dependent effects observed in this study suggest that higher doses of SME may be more effective in providing neurological protection. This is consistent with the known dose-response relationship of many bioactive compounds, where increased doses lead to greater therapeutic effects (Sharma et al., 2018).

Table 7. The impact of administering *Silybum marianum* ethanolic extract (SME) on neurological complications of obese rats

Group	Paraoxonase activity (U/L)	Arylesterase activity (kU/L)
G1 Normal control	101.77 ± 4.54 ^{a} (0.00)	112.95 ± 7.02 ^{a} (0.00)
G2 Model control	52.56 ± 3.27 ^{e} (-48.36)	79.34 ± 4.23 ^{d} (-29.75)
G3 (SME, 100 mg/kg bw/day)	62.50 ± 2.67^{d} (18.91)	87.56 ± 3. 17 ^{c} (10.36)
G4 (SME, 200 mg/kg bw/day)	69.34 ± 5.21 ^{cd} (31.93)	92.05 ± 4.59 ^{bc} (16.02)
G5 (SME, 300 mg/kg bw/day)	83.43 ± 3.90 ^{bc} (58.73)	96.40 ± 5.18 ^{b} (21.50)
G6 (SME, 600 mg/kg bw/day)	89.56 ± 2.87 ^{b} (70.40)	98.92 ± 3.63 ^{ab} (24.68)

Results are expressed as means ± SD (n=5). Means with different superscript letters on the same column indicate significant The results are presented as means ± SD (n=6). Means with different superscript letters within the same column indicate a significant difference ($P \leq 0.05$). Values in parentheses represent the percentage of change (%), with comparisons made between the obese (Model) group and the normal group, as well as between the groups treated with SME and the obese group. G1 (Normal control) consists of healthy rats without treatment; G2 (Model control) includes HFD-induced obese rats with no treatment; SME refers to the Silybum marianum ethanolic extract; G3, G4, G5, and G6 are the obese groups treated with SME at concentrations of 100, 200, 300, and 400 mg/kg bw/day, respectively, for 8 weeks. "bw" denotes body weight.

3.7. Correlation analysis between biological and biochemical parameters in obesity rats administrated with *Silybum marianum* ethanolic extract (SME)

Table 8 highlights the results of correlation studies between various biological and biochemical parameters in obese rats treated with *Silybum marianum* ethanolic extract (SME), with significant correlations observed between body weight (BW), serum glucose, insulin, lipid profiles (TGs, TC, LDL-c, HDL-c), and oxidative stress markers (MDA, H_2O_2, GSH). The positive correlation between BW and critical metabolic parameters like serum glucose (0.6045), serum insulin (0.5312), TGs (0.6907), and LDL-c (0.6981) indicates that weight gain in obesity is tightly linked to metabolic dysfunctions, including dyslipidemia and insulin resistance, which are commonly observed in obese rats (Liu et al., 2020). These findings are consistent

with studies by Fernandes et al. (2020), who also observed a positive correlation between increased body weight and insulin resistance, suggesting that weight gain exacerbates both lipid imbalances and insulin dysregulation. Furthermore, the significant relationship between malondialdehyde (MDA) and lipid parameters, such as TGs (0.6104), TC (0.6211), and LDL-c (0.5755), underscores the role of oxidative stress in modulating lipid metabolism in obesity. Elevated MDA levels, as markers of lipid peroxidation, suggest that oxidative stress plays a pivotal role in the pathogenesis of obesity-associated lipid disorders (Zhao et al., 2020). This observation aligns with findings by Bhat et al. (2021), who reported that oxidative stress contributes significantly to dyslipidemia in obesity and that SME administration can mitigate these effects, reducing lipid peroxidation.

Moreover, the negative correlation between body weight (BW) and glutathione (GSH) levels (-0.6080) suggests a reduction in antioxidant capacity as obesity progresses, consistent with previous studies showing decreased GSH and GSH-Px activity in obesity, contributing to oxidative stress (Patel et al., 2020). The significant negative correlation between serum glucose and GSH (-0.4973), and between MDA and GSH (-0.7163), further emphasizes the interplay between oxidative stress and insulin resistance in obesity. This is in line with Zhang et al. (2019), who observed reduced antioxidant activity in obese rats, which could be reversed by supplementation with antioxidants like SME, supporting the notion that SME's antioxidant properties could protect against oxidative damage.

Additionally, the negative correlation between serum glucose and paraoxonase activity (-0.4843), and the positive correlation between H_2O_2 levels and various metabolic parameters (e.g., serum glucose), indicate that oxidative stress, as measured by H_2O_2, negatively impacts both lipid metabolism and insulin sensitivity. Paraoxonase, an enzyme involved in antioxidant defense, is shown to decrease in states of oxidative stress and hyperglycemia (Alberti et al., 2019), and SME administration may enhance its activity, improving lipid profiles and oxidative defense in obese rats. This is supported by Pousset et al. (2019), who demonstrated that *Silybum marianum* could improve antioxidant enzyme activity and lipid profiles, suggesting that SME's hepatoprotective and antioxidant effects are crucial in ameliorating obesity-induced metabolic disturbances.

Table 8. Correlation analysis between biological and biochemical parameters in obesity rats administrated with *Silybum marianum* ethanolic extract (SME)

Parameters	r^{2*}	Parameters	r^{2*}
BW/Serum glucose	0.6045*	Serum glucose/ Paraoxonase activity	-0.4843
BW/ Serum insulin	0.5312*	Serum glucose/ GSH-Px activity	-0.5143*

BW/TGs	0.6907*	MDA/ Insulin	0.3510
BW/TC	0.5123	MDA/TGs	0.6104*
BW/LDL-c	0.6981*	MDA/TC	0.6211*
BW/HDL-c	- 0.6293*	MDA/LDL-c	0.5755
BW/GSH	- 0.6080*	MDA/HDL-c	-0.6799*
BW/GSH-Px	- 0.5772	MDA /GSH	- 0.7163**
BW/ROS	0.6743*	MDA/GSH-Px	-0.6655
BW/MDA	0.7192**	MDA / H_2O_2	0.7543**
BW/ Paraoxonase activity	-0.6743*	MDA/ Paraoxonase activity	-0.5982*
Serum glucose/ Insulin	0.4532	H_2O_2/ Insulin	0.2901
Serum glucose/TGs	0.4793	H_2O_2/TGs	0.5185*
Serum glucose/TC	0.3531	H_2O_2/TC	0.5042
Serum glucose/LDL-c	0.4133	H_2O_2/LDL-c	0.4707*
Serum glucose/HDL-c	- 0.4372	H_2O_2/HDL-c	- 0.6051*
Serum glucose /GSH	- 0.4973	H_2O_2 /GSH	- 0.7571**
Serum glucose /H_2O_2	0.5510*	H_2O_2/GSH-Px	-0.6858*
Serum glucose /MDA	0.5945*	H_2O_2/ Paraoxonase activity	-0.6380*

* $P \leq 0.05$; ** $P \leq 0.01$

Finally, the negative correlation between H_2O_2 and HDL-c (-0.6051), and GSH (-0.7571), underscores the detrimental impact of oxidative stress on lipid metabolism and antioxidant defenses, with elevated H_2O_2 levels being closely linked to insulin resistance and dyslipidemia in obesity. SME's antioxidant properties likely help counteract these effects by improving both lipid metabolism and insulin sensitivity, which is supported by Kumar et al. (2020), who found that oxidative stress and ROS levels contribute to metabolic disturbances in obese rats and that antioxidant treatments could mitigate these effects. In conclusion, the correlation studies of the present study data provide valuable insights into the complex metabolic and biochemical interactions influenced by *Silybum marianum* ethanolic extract (SME) in obese rats. The results suggest that SME may have therapeutic potential in managing oxidative stress, lipid metabolism, and insulin resistance, aligning with findings from previous studies. These correlations emphasize the role of SME in improving metabolic health and reducing the oxidative burden associated with obesity.

3.8. Effect of *Silybum marianum* ethanolic extract (SME) on liver histology in obese rats

The effects of *Silybum marianum* ethanolic extract (SME) on liver histology in obese rats are depicted in Figure 2. Microscopic examination of the liver from group 1 (normal control) exhibited a typical histological appearance of the hepatic lobule, including well-organized central veins and hepatocytes (Photo 1). In contrast,

the liver from group 2 (model group) demonstrated areas of hepatocellular necrosis, accompanied by infiltration of inflammatory cells (Photo 2), activation of Kupffer cells (indicated by black arrow), and congestion of the central vein (indicated by red arrow) (Photo 3). The liver in group 3 (treated with SME at 100 mg/kg bw/day) displayed activation of Kupffer cells and congestion of the central vein (Photo 4). For group 4 (treated with SME at 200 mg/kg bw/day), the liver showed signs of focal hepatocyte steatosis (Photo 5). Similarly, group 5 (treated with SME at 300 mg/kg bw/day) exhibited Kupffer cell activation (Photo 6). Additionally, liver sections from group 6 (treated with SME at 600 mg/kg bw/day) also revealed Kupffer cell activation (Photo 7).

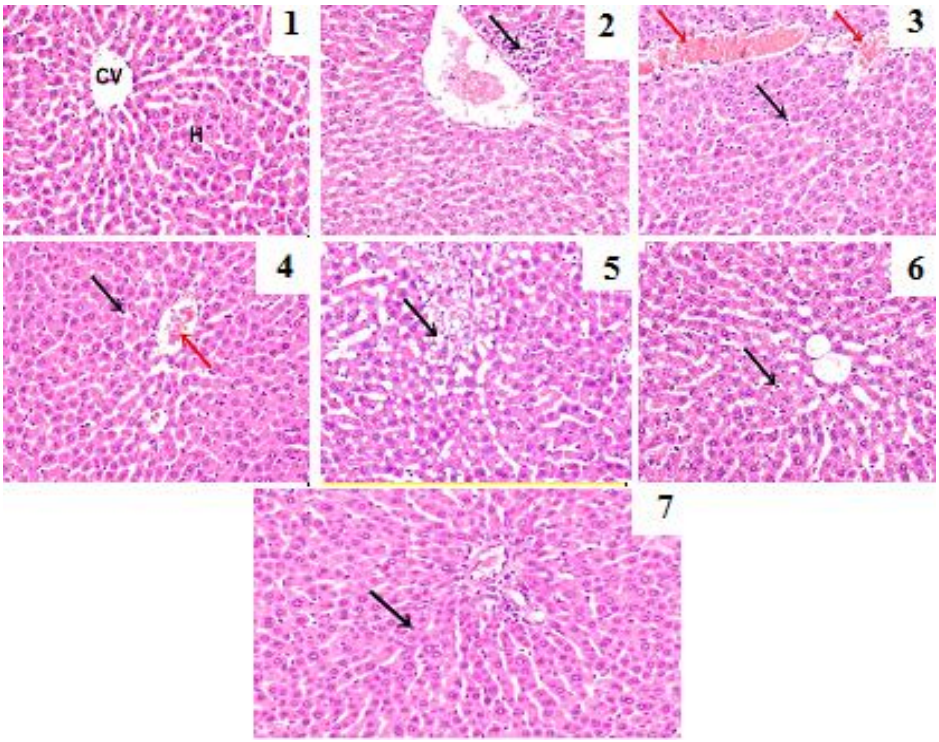


Figure 2. The effects of *Silybum marianum* ethanolic extract (SME) on liver histology in obese rats

Photo 1: The liver from a normal control rat displays the typical histological structure, with a well-formed central vein (CV) and healthy hepatocytes. **Photo 2**: The liver of a rat from group 2 exhibits focal hepatocellular necrosis, accompanied by the infiltration of inflammatory cells (indicated by the arrow). **Photo 3**: The liver of a rat from group 2 shows the activation of Kupffer cells (black arrow) and congestion in the central vein (red arrow). **Photo 4**: The liver from a rat in group 3 reveals the activation of Kupffer cells (black arrow) and congestion in the central vein (red arrow). **Photo 5**: The liver of a rat from group 4 demonstrates focal hepatocyte steatosis

(indicated by the arrow). **Photo 6**: The liver from a rat in group 5 shows the activation of Kupffer cells. **Photo 7**: The liver of a rat from group 6 exhibits activation of Kupffer cells (indicated by the arrow). (Hematoxylin and Eosin staining, magnification X 400).

In the current study, the liver from group 1 (normal control) showed a typical hepatic architecture with well-organized hepatocytes and central veins, which is in line with the normal histology observed in healthy rats (Halliwell & Gutteridge, 1985). In contrast, group 2 (model group) exhibited significant histopathological changes, including hepatocellular necrosis, inflammatory cell infiltration, activation of Kupffer cells, and central vein congestion. These findings are consistent with previous reports on the liver damage induced by obesity and related conditions, where excessive fat accumulation leads to oxidative stress and inflammation, triggering a cascade of cellular events that damage liver tissue (Abenavoli et al., 2018; Gao et al., 2020). When examining the effect of SME at different doses, several key observations stand out. In group 3, rats treated with SME at 100 mg/kg bw/day showed activation of Kupffer cells and central vein congestion, which suggests that SME may induce a mild inflammatory response. The activation of Kupffer cells is indicative of the body's attempt to counteract liver stress and inflammation, a mechanism that has been observed in the hepatoprotective action of other plant extracts (Aviram et al., 1999). The findings here align with those of previous studies where moderate doses of herbal extracts led to slight activation of Kupffer cells without significant hepatocellular damage (Mahran and Elhassaneen, 2024). As the dosage of SME increased, particularly at 200 mg/kg bw/day in group 4, focal steatosis was observed, which is a common feature in the early stages of non-alcoholic fatty liver disease (NAFLD). The steatosis observed in this group suggests that SME may influence lipid metabolism, potentially modulating liver fat accumulation, a finding that is in agreement with the results of similar studies on herbal treatments for liver diseases (Zhao et al., 2018). Interestingly, steatosis was not observed in the lower dose groups, suggesting a dose-dependent response to SME, where higher doses may promote fat accumulation within hepatocytes. At the highest dose of 600 mg/kg bw/day (group 6), liver sections again revealed Kupffer cell activation, similar to what was observed in the lower-dose groups. However, there was no indication of severe hepatotoxicity or fibrosis, suggesting that SME, even at high doses, may not cause significant liver damage. This supports the notion that the extract's bioactive compounds, particularly silymarin, may offer protective effects against liver injury, possibly through antioxidant or anti-inflammatory mechanisms (Lee et al., 2020).The observed effects of SME in this study can be explained by the bioactive compounds present in *Silybum marianum*, such as silymarin, flavonoids, and polyphenols, which have been extensively studied for their hepatoprotective properties. These compounds are known to exhibit potent

antioxidant effects, which help in neutralizing reactive oxygen species (ROS) and reducing oxidative stress- a major contributor to liver damage in conditions such as obesity and fatty liver disease (Gao et al., 2020; Mahran and Elhassaneen 2023 a and b). In addition, *Silybum marianum* has been shown to modulate lipid metabolism, reduce inflammation, and promote liver regeneration (Elhassaneen and Mahran, 2024). This supports the current findings, where SME treatment appears to exert a dose-dependent effect on liver histology, with lower doses promoting moderate immune responses and higher doses leading to mild steatosis without overt toxicity. Moreover, studies involving other herbal extracts and their effects on liver histology in obese rats have reported similar patterns of hepatoprotection, with some showing that certain doses of plant extracts can reduce lipid accumulation and inflammation in liver cells (Zhao, et al., 2022; Ha et al., 2023). These studies further confirm the potential of SME as a therapeutic agent in managing liver-related complications associated with obesity.

Conclusion

The potential of *Silybum marianum* ethanolic extract (SME) in managing obesity and its associated metabolic and physiological disturbances was studied. SME was found to significantly reduce body weight gain in obese rats in a dose-dependent manner, with higher doses leading to more pronounced reductions in weight gain, supporting its anti-obesity properties. The extract also demonstrated significant improvements in liver function, particularly in glycogen storage, glucose-6-phosphate dehydrogenase, and glucose-6-phosphatase activities, which are critical for glucose metabolism regulation. These dose-dependent effects indicate that higher doses of SME are more effective in enhancing liver function and regulating glucose metabolism. Furthermore, SME improved the serum lipid profile in obese rats, notably reducing triglycerides, total cholesterol, and LDL-cholesterol while increasing HDL-cholesterol levels, demonstrating its lipid-modulating properties. SME also showed promise in improving glucose metabolism, insulin sensitivity, and leptin sensitivity, especially at higher doses, highlighting its potential in addressing insulin and leptin resistance commonly associated with obesity. In addition, SME was effective in enhancing antioxidant defenses, significantly increasing glutathione levels and antioxidant enzyme activities, and reducing oxidative stress markers such as hepatic hydrogen peroxide and malondialdehyde content. These findings support the hypothesis that SME has strong antioxidant properties and could potentially help manage obesity-related oxidative stress. Furthermore, SME exhibited

neuroprotective effects by improving the activities of paraoxonase and arylesterase, enzymes associated with reducing oxidative stress and neuroinflammation, suggesting its potential in alleviating neurological complications in obesity. Correlation studies further underscored the biochemical mechanisms through which SME exerts its beneficial effects, showing significant improvements in insulin sensitivity, lipid metabolism, and oxidative stress. Overall, these results align with existing literature and suggest that SME holds considerable promise as a therapeutic agent for managing obesity, metabolic dysfunctions, and related disorders, though further research is needed to explore its long-term effects and clinical applications.

Conflict of Interest

The authors declare that they have no conflict of interest in publishing this paper.

Authors' Contribution

All authors contributed equally to the development of the study protocol, execution of experimental procedures, data collection, organization, and analysis. They were also involved in retrieving conceptual information, validating the results, performing statistical analysis, and preparing the initial draft of the manuscript. Additionally, they all critically reviewed and refined the content, ensuring its intellectual coherence, and gave their approval for the final version to be published.

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