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# Bioactive compounds and antioxidant activities of milk thistle (*Silybum marianum*) extract and their potential roles in the prevention of diet-induced obesity complications

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**Abstract:** The present study aims to determine the bioactive compounds and biological activities of the *Silybum marianum* ethanolic extract (SME). Also, the potential effects of SME on obesity and other related complications in rats will be investigated. Data of the proximate chemical composition of *Silybum marianum* seed powder indicated that carbohydrates were the largest compound (67.21%) followed by ash (16.23 ± 1.14 %), crude fat (26.72%), Total protein (22.17%), crude fiber (7.17%) and ash (2.83%). Also, bioactive compounds content of SME indicated that Silymarin was the most largest compound (269.65 mg. $g^{-1}$) followed by phenolics (127.65 mg gallic acid equivalent. $g^{-1}$), flavonoids (65.1 mg quercetin equivalent. $g^{-1}$), tannins (39.49 mg catechine equivalent. $g^{-1}$), α-tocopherol (27.43 mg. $g^{-1}$), chlorophyll (11.54 mg. $g^{-1}$), β-carotene (6.83 mg. $g^{-1}$) and anthocyanin's (4.29 mg Cyanidin 3-glucoside, CCy3G equivalent.$g^{-1}$). The samples also exhibited several high biological activities which include inhibition of low density lipoprotein (LDL) oxidation and scavenging of free radicals. Such important biological effects could play important roles in strategies to combat / treat obesity and its complications in rats. SME intervention by 200 to 800 mg/kg bw/day by oral gavages for 8 weeks consecutive days leads to significantly ($p \leq 0.05$) decrease the body weight, lower the serum lipid profile parameters (TGs, TC and LDL-c), enhanced the liver functions in obese, and positively manipulate of the obesity-related the histopathological changes of the model (obese) control group. Therefore, data of the present study recommended like of that *Silybum marianum* extracts to be included in our daily diets, drinks, food supplementation and pharmacological formulae for the obese patients.

***Keywords:*** *Silybum marianum, inhibition of LDL oxidation, free radicals scavenging activity, bogy weight, serum lipid profile, liver functions*

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## 1. Introduction

Obesity may be defined as a state of imbalance between calories ingested versus calories expended which would lead to excessive or abnormal fat accumulation [1]. It is results from inherited, physiological and environmental factors, combined with diet, physical activity and exercise choices. The prevalence of overweight and obesity is commonly assessed by using body mass index (BMI), which defined as the weight in kilograms divided by the square of the height in meters ($kg/m^2$) [2]. A BMI over 30 $kg/m^2$ is defined as obese. These markers provide common benchmarks for assessment, but the risks of disease in all populations can increase progressively from lower BMI levels [3,4]. Annually, about 4.7 million premature deaths occur due to obesity. It was ranked fifth among the leading preventable causes of death, making up 12.3% of all *deaths* worldwide and *8.4%* of total disability-adjusted life years (DALYs) lost to non-communicable diseases [5,6]. Also, Ramasamy et al., [7] stated that the medical and psychological complications of obesity participate in a large share of health care expenditures, and also generate additional economic costs on the general budgets of countries through loss of worker productivity, increased disability, and premature loss of individuals. Perhaps these problems come through the association of obesity with various diseases, particularly cardiovascular diseases, diabetes mellitus type 2, obstructive sleep apnea, certain types of cancer, osteoarthritis, asthma, and neurological and immunological disorders [3,8-16]. These co-morbidities are most commonly shown in metabolic syndrome, a combination of medical disorders which includes: diabetes

mellitus type 2, high blood pressure, high blood cholesterol, and high triglyceride levels [15,17-19]. Also, obesity-induced insulin resistance seems to be related to chronic inflammation, glucotoxicity, lipotoxicity, and adipokine dysregulation [20]. These obesity comorbidities are also more commonly seen in metabolic syndrome, a group of medical disorders that includes: type 2 diabetic mellitus, and high blood pressure, cholesterol, and triglyceride levels.

In obesity treatment/prevent strategies, a number of pharmacological approaches have been investigated in recent years but few therapeutically effective and safe products have been developed [21]. Also, the epochal pharmacological therapy is costly and associated with multiple side effects resulting in patient non-compliance. Hence, there was an urgent need to search for alternative therapies especially from natural sources, as they are cost effective and have few side effects. In this direction, several plant parts have been applied as anti-obesity agents in our previous study [12,13,15,16,22,23]. These plant parts initially took the form of crude drugs such as powders, extracts and other herbal formulations which exhibited positively effects in preventing and/or treating obesity and its complications in experimental animals. Therefore, the results of these studies were an encouraging factor to expand in this field, using different plant parts that are widespread in the global and local environment.

Milk thistle (*Silybum marianum* L., Family: *Asteraceae*) is an annual/biennial plant, native of Mediterranean area and now growing and cultivated worldwide including Egypt [25-27] (Figure 1). It has been used for centuries in medicine, mainly to treat kidney, spleen and liver diseases [28]. Such as reviewed by Abenavoli et al., [29], the Roman, Greek, German, England and American physician, pharmacologist and naturalist in the old ages used this plant juices, extracts and prescriptions to carry off the bile, against serpent bites and all melancholy diseases, treat liver ailments, cure fever, and recommended for varicose veins, menstrual problems, and congestion of the spleen, kidney and liver. Also, milk thistle fruits contain several bioactive compounds, mainly, silymarin. Silymarin is a complex mixture of polyphenolic molecules, including seven closely related flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin) and one flavonoid (taxifolin) [30]. Silibinin, a semipurified fraction of silymarin, is primarily a mixture of two diastereoisomers, silybin A and silybin B, in a roughly 1:1 ratio [31]. Traditional milk thistle extract is made from the seeds, which contain approximately 4–6% silymarin [32].The extract consists of about 65–80% silymarin and 20–35% fatty acids, including linoleic acid. Regarding the beneficial medical properties of milk thistle and main constituent, silymarin, several animal and human studies have been suggested that silymarin could be a good candidate in the therapy of metabolic syndrome disease [33-36]. Metabolic syndrome is the medical term for a combination of diabetes, high blood pressure (hypertension) and obesity which put the subjects at greater risk of getting coronary heart disease, stroke and other conditions that affect the blood vessels [37,27]. Also, different pharmacological functions of silymarin in liver diseases: antioxidant, antifibrotic regenerative (stimulate hepatic regeneration, choleretic, hepatoprotective, immunostimulating, and anti-inflammatory were also reported [27,38]. Thus, milk thistle is among the top - selling herbal dietary supplements all over the world [39].

All the literature data suggest that although preclinical data are encouraged, more well-designed trials are needed to fully understand the real value of milk thistle seeds extracts (MTE) in obesity and its related complications. Complications attributable to obesity create a huge economic, humanistic, and clinical burden all over the world. Reducing obesity could help dramatically decrease the catastrophic health effect of these diseases which in turn decreases mortality and disability-adjusted life years (DALYs) lost. Therefore, the current study aims to prepare the ethanolic extract from the seeds of milk thistle (*Silybum marianum* L.) and study its bioactive compounds content and antioxidant activities. Also, the potential protective effects of such extract on obesity and its complications in experimental rats will be in the scope of this investigation.


**Figure 1.** Milk thistle (*Silybum marianum* L.) growing wild on the sides of irrigation canals in Mit Ghorab village, ElSinbellaween Center, Dakahlia Governorate, Egypt: Leaves, flowers, mature fruits, seeds and ethanolic extract

# 2. Materials and Methods

## 2.1. Materials

### 2.1.1. Milk thistle, (*Silybum marianum* L.) fruits

Dried fruits of wild plant populations growing in public irrigation canals were collected with special arrangements from Mit Ghoarb Village of, Sinbillawin, Dakahlia Governorate, Cairo Egypt. The fruits were collected in burlap sacks, and transported to the Agricultural Plant Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt for taxonomic confirmation.

### 2.1.2. Diet-induced obesity (DIO)

Diet-induced obesity (DIO), product no. D1245, was purchased from Research Diets, Inc. NJ, Protein, 24 g/100 g (Kcal, 20%) carbohydrates, 41 g/100 g (Kcal, 35%) and fat, 24g/100 g (Kcal, 45%).

### 2.1.3. Chemicals

Bioactive compounds standard [gallic acid (GA), catechine (CA), α-tocopherol and Butylated hydroxytoluene (BHT)], DDPH (2,2-diphenyl-1-picrylhydrazyl), AAPH [2,2′-Azobis(2-methylpropionamidine) dihydrochloride], dimethyle sulfoxide (DMSO),silymarin, β-carotene, α-tocopherol and sulphanilic acid, were purchased from Sigma Chemical Co., St. Louis, MO.All other chemicals (Except as otherwise stated), reagents and solvents were of analytical grade were purchased from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt.

### 2.1.4. Kits

Kit's assays for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), malondialdehyde (MDA), serum lipids profile (triglycerides, TGs; total cholesterol, TC; high density lipoprotein cholesterol, HDL-c), serum glucose and insulin level were purchased from BIODIAGNOSTIC, Dokki, Giza, Egypt. Reduced glutathione (GSH) and oxidized glutathione (GSSG) were assayed by the kits provided by MyBioSource, Inc., San Diego, CA, USA.). Reactive oxygen species (ROS) assayed by the kits was provided by Sigma-Aldrich, St. Louis, Missouri, USA. Casein was obtained from Morgan Chemical Co., Cairo, Egypt. Vitamins and salts mixtures in food grade, organic solvents and other chemicals in analytical grade were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

### 2.1.5. Machines

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

## 2.2. Methods

### 2.2.1. Preparation of *Silybum marianum* seeds ethanol extracts (SME)

Dried fruits of *Silybum marianum* samples were transferred to the laboratory. The seeds were extracted manually and sieved to remove foreign bodies and damaged seeds. Seed were ground in high miller speed (Moulinex Egypt, Al-Araby Co., Egypt) and reduced to powder (20 mesh) and mixed to obtain homogeneous samples. SMEE was prepared such as mentioned in Oludemi et al., [40]. Five grams of *Silybum marianum* dried powder were extracted in a Soxhlet apparatus (Soxhlet Semiautomatic apparatus Velp company, Italy) for 5-6 h (25 ± 5 min per cycle) using 80% ethanol. Finally, the solvent was evaporated under reduced pressure (rotary evaporator Büchi R-210, Switzerland) to obtain the dried solvent extract and stored at 4 $^{0}$C before use. The total yield of SME was 5.61% (w/w) in terms of the *Silybum marianum* seeds.

### 2.2.2. Chemical analysis of *Silybum marianum* seeds powder

BA samples were analyzed for proximate chemical composition including moisture, protein (T.N. × 6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy), fat (Soxhlet Semiautomatic apparatus Velp company, Italy, petroleum ether solvent), ash, fiber and dietary fiber contents were determined using the methods described in the AOAC, (1995). Carbohydrates calculated by differences: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber)

### 2.2.3. Bioactive compounds determination in *Silybum marianum* seeds ethanol extracts (SME)

Total phenolics were determined using Folin-Ciocalteu reagent according to Singleton and Rossi, [41] and Wolfe et al., [42]. Results are expressed as gallic acid and equivalents (GAE). Total flavonoids contents were estimated using colorimetric assay described by Zhisen et al., [43]. Total flavonoid contents were expressed as catechin equivalent, CAE. Total content of anthocyanins in the sample was measured spectrophotometrically such as described by Sukwattanasinit et al., [44] using molar extinction coefficient of cyanidin-3,5-diglucoside (26 300 $M^{-1}$ $cm^{-1}$). Tannins were determined in samples by the method of Van-Burden and Robinson [45] and expressed as mg catechine per g of dw. Silymarin was determined spectrophotometrically method such as described by Rajasekaran et al., [46]. The method is based on the reaction of silymarin with diazotized sulphanilic acid in alkaline medium to form red color chromogen which exhibiteds absoption maximum at 460 nm. The total chlorophyll (a+b) concentration was determined spectrophotometrically according to the method of Zilha et al., [47] by measuring the absorbance of the extract at 662 nm and 644nm, respectively. The resulting absorbance measurements are then applied to a standard equation. β-carotene was determined by UV-Vis

spectrophotometric method described by Biswas et al., [48]. α-tocopherol was determined according to the method of Tütem et al., [49] by using of the copper (II)-neocuproine spectrophotometric method.

#### 2.2.4. Antioxidant activity determination

##### 2.2.4.1. DPPH radical scavenging assay

Free radical scavenging ability of brown algae (*Sargassum subrepandum*) extracts was tested by DPPH radical scavenging assay as described by Desmarchelier et al. [50]. A solution was prepared, and 2.4 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM in methanol) was mixed with 1.6 mL of *G. lucidum* extract at different concentrations (12.5–150 µg/mL). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm (UV-160A; Shimadzu Corporation, Kyoto, Japan). BHT was used as reference. Percentage DPPH radical scavenging activity was calculated by the following equation: DPPH radical scavenging activity (%) =[(A0− A1)/A0]×100. where $A_0$, absorbance of the control, and $A_1$, absorbance of the BA / BHT. Then inhibition (%) was plotted against concentration, and $IC_{50}$ was calculated from the graph.

##### 2.2.4.2. Inhibition of low density lipoprotein (LDL) oxidation

Inhibition of LDL oxidation was determined in brown algae (*Sargassum subrepandum*) according to the method of Princen *et al.*, [51]. Adult male white albino rat, Sprague Dawley strain, serum was collected and diluted by phosphate buffer (50 mM, pH 7.4) to the concentration of 0.6%. Quantities of 5.0 ml diluted serum were mixed with 10 µl DMSO or 10 µl DMSO containing various concentrations of the all tested algae extracts. A 20 µl of CuSO4 solution (2.5 mM) was added to initiate the reaction and the absorbance at 234 nm was recorded then was taken every 20 min thereafter for 140 min at room temperature. The final result was expressed by calculation the net area under the curve.

### 2.3. Biological experimental

#### 2.3.1. Ethical approval

Biological experiments for this study were ethically approved by the Scientific Research Ethics Committee (Animal Care and Use), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval no. 21- SREC- 03-2022).

#### 2.3.2. Animals

Animals used in this study, adult male albino rats (146.81±5.95 g per each) were obtained from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

#### 2.3.3. Standard/Basal Diet (BD)

The basic diet prepared according to the following formula as mentioned by Reeves et al., [52] as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamin and minerals mixtures component were formulated according to Reeves et al., [52].

#### 2.3.4. Experimental design

All biological experiments performed a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council [53]. Rats (n=36 rats), were housed individually in wire cages in a room maintained at 25 ± 3 $^0$C, relative humidity (56±4%), a 12-hr lighting cycle and kept under normal healthy conditions. All rats were fed on BD for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group, normal control, (Group 1, 6 rats) still fed on BD and the other main group (30 rats) was used for obesity induction by feed with diet-induced obesity (DIO) for 8 weeks and classified into five sub groups as follow: group (2), model control, fed on BD only as a positive control (rats with obesity) and groups (3-6) fed on BD and administered by oral gavages, using a feeding needle with 200, 400, 600, and 800 mg/kg bw/day SMEE, respectively. SMEE extract concentrations were selected for experiments based on many of the results of previous studies [54]. Each of the above groups was kept in a single cage for 8 weeks. Rats were weighted at the beginning of experimental then weekly and at the end of the experimental period.

#### 2.3.5. Blood sampling

At the end of experiment period, 4 weeks, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum according to Drury and Wallington, [55]. Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20$^o$C until analysis.

#### 2.3.6. Hematological analysis

Different tested parameters in serum were determination using specific methods as follow: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities according to Yound, [56], Tietz, [57], and Yound, [56], respectively. Enzymatic determination of serum glucose was carried out colorimetrically according to Yound, [56] and Tietz, [57]. Insulin level in pkasma was determined according to the method of Held [58]. Triglycerides (TGs), Total cholesterol (TC) and High density lipoprotein-cholesterol (HDL-c) determined in serum according to the methods of Fossati and Prenape [59], Richmod [60], and Lopes-Virella et al., [61], respectively. Low density lipoprotein cholesterol (LDL-c) was assayed according to the equation of Fniedewald et al., [62] as follow: LDL-c = TC – (HDL-c + TGs/5). Glutathione fractions (GSH and GSSG) were measured colorimetrically in serum samples such as described by Ellman, [63]. Serum malonaldialdehyde (MDA) content was measured by the thiobarbituric acid (TBA) method according to the methods of Buege and Aust, [64].

Reactive oxygen species (ROS) was determined by a colorimetric method described by Erel, [65].

### 2.4. Statistical Analysis

Results were expressed as means ± SD with Microsoft Excel Software (version 15.0, 2013). The data were analyzed statistically using Student t-test and MINITAB 12 computer program statistical software (Minitab Inc., State College, PA). A value of $P \leq 0.05$ was considered as statistically significant.

## 3. Results and Discussion

### 3.1. Chemical studies

#### 3.1.1. Proximate chemical composition of *Silybum Marianum* seeds

The proximate chemical composition of *Silybum marianum* seeds was showed in Table 1. From such table it could be noticed that SMS contained a high percentage of carbohydrates (67.21%) while crude fat/oil, crude protein, crude fiber and ash contents were 26.72, 22.17, 7.17 and 2.83%, respectively. Data of the present study were partially similar to obtained by several previous studies. For example, four varieties of *Silybum marianum* in Iran were studied and the oil content of the seeds ranged from 26-31 g/100g [66]. Also, Harrabia *et al*., [67] reported the level of total lipid (g/100 g of the dry weight of seeds) was 30.5% in the milk thistle seed growing wild in Tunisi while was recorded 21% in China [68]. Recently, Mahran and Elhassaneen [69] found that *Silybum marianum* seeds contained a high percentage of crude fat/oil (28.04%), while crude protein, crude fiber, ash and total carbohydrates contents were 23.85, 6.50, 2.47 and 31.58 %, respectively. From all the above studies and others, it could be concluded that the chemical composition of *Silybum marianum* seeds vary with one or combination of the following factors, genotype and environmental conditions (season, geographical location, drought stress and duration etc., [70,71]. But any way, all of data suggested that *Silybum marianum* seeds may serve as a natural promise innovative source of different nutrients.

**Table 1. Proximate chemical composition of *Silybum marianum* seeds**

| Component (g/100g) | Amount |
|---|---|
| Moisture | 8.32 ± 1.02 |
| Crude fat | 26.72 ± 0.87 |
| Crude protein | 22.17 ± 0.98 |
| Crude fiber | 7.17 ± 0.77 |
| Ash | 2.83 ± 0.32 |
| Carbohydrates | 67.21 ± 1.12 |

Data expressed as the mean value of three replicates ± SD.

#### 3.1.2. Bioactive components of *Silybum marianum* extract (SME)

*Silybum marianum* seeds ethanolic extract (SME) contained a wide range of bioactive compounds such as shown in Table 2. From such table it could be noticed that silymarin was the largest compound (269.65 ± 4.27 mg. $g^{-1}$) followed by total phenolics (127.65 ± 6.59 mg gallic acid equivalent. $g^{-1}$), flavonoids (65.1 ± 2.71 mg quercetin equivalent. $g^{-1}$), tannins (39.49 ± 2.54 mg catechine equivalent. $g^{-1}$), α-tocopherol (27.43 ± 1.26 mg. $g^{-1}$), chlorophyll (11.54 ± 1.88 mg. $g^{-1}$) and anthocyanin's (4.59 ± 0.82 mg Cyanidin 3-glucoside, CCy3G equivalent.$g^{-1}$). In similar study, Mahran and Elhassaneen, [69] *Silybum marianum* seeds oil contained a high percentage of several bioactive compounds including total phenolics, flavonoids, silymarin, β-carotene and play a predominant role in the antioxidant capacity of the SME. For example, several studies have reported that phenolic compounds including flavonoids act as antioxidative, anticancer, antibacterial, cardio-protective, anti-inflammatory, and immune-stimulating agents [27,72,73]. Also, silymarin found in MSE knows to reduce the oxidative stress and subsequent cytotoxicity which protecting cells/liver cells which have not yet been irreversibly damaged [74]. As a result of its antioxidant and anti-inflammatory properties, silymarin exhibited hepatoprotective effects i.e. protecting the liver from dietary steatosis, oxidative stress, and food contaminants induced liver injury [54,75-78]. Furthermore, α-tocopherol is the most biologically active member in the vitamin E family and is a significant compound SME and very essential lipid oxidation inhibitor in biological systems and food [79-81]. Additionally, chlorophyll and carotenoid pigments are important because of their nutritional and technological properties, especially their anti- and pro-oxidant properties [82]. On the other side, bioactive compounds reported in SME i.e. Phenolics, flavonoids, carotenoids and anthocyanin's play important biological roles in preventing and/or treating many diseases such as diabetes, atherosclerosis, cancer, obesity, bone, anemia and aging [13,54,83-85].

**Table 2. Bioactive components of *Silybum marianum* extract (SME)**

| Components | Amount |
|---|---|
| Total phenolics (mg gallic acid equivalent. $g^{-1}$) | 127.65 ± 6.59 |
| Flavonoids (mg quercetin equivalent. $g^{-1}$) | 65.1 ± 2.71 |
| Silymarin (mg. $g^{-1}$) | 269.65 ± 4.27 |
| α-tocopherol (mg. $g^{-1}$) | 27.43 ± 1.26 |
| β-carotene (mg. $g^{-1}$) | 6.83 ± 0.99 |
| Chlorophyll (mg. $g^{-1}$) | 11.54 ± 1.88 |
| Tannins (mg catechine equivalent. $g^{-1}$) | 39.49 ± 2.54 |
| Anthocyanin's (mg Cyanidin 3-glucoside, CCy3G equivalent.$g^{-1}$). | 4.59 ± 0.82 |

Data expressed as the mean value of three replicates ± SD.

#### 3.1.3. Antioxidant capacity of *Silybum marianum* seeds ethanolic extract (SME) DPPH radical scavenging activity

The free radical scavenging activity (FRSA, %) of *Silybum marianum* extract (SME) and standard (BHT) are shown in Figure 2 and Table 3. Such data indicated that, SME possessed the highest activity compared to the BHT. At a concentration of 100 µg/mL, the radicals scavenging activity of SME and BHT were 86.87 and 91.24%, respectively. For the IC50, SME and BHT were recorded 11.61± 0.70 and 8.21± 0.18 µg/mL, respectively. The free radical scavenging activity of SME and BHT was in the following order: standard (BHT) > SME**.** In general, the theory of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals scavenging activity assay is based on measurement of the diene conjugation by absorption at 234 nm in the presence of DDPH substrate is commonly used for determining the oxidative stability of the SME samples [86]. Such assay used successfully to evaluate the

antioxidant activity/oxidative stability of different plant parts including vegetables, fruits, algae, food processing by-products etc., [85,87,88]. Several previous studies reported that the FRSA are very important to prevent the adverse effects of free radicals in many diseases including obesity, diabetes, cancer, cardiovascular, neurological, pulmonary, nephropathy and aging diseases. Data of present study suggest that SME showed free radical scavenging activity which due to their high content of different categories of bioactive compounds (antioxidants) including Silymarin, phenolics, flavonoids, tannins, α-tocopherol, carotenoids, chlorophyll and anthocyanin's etc.

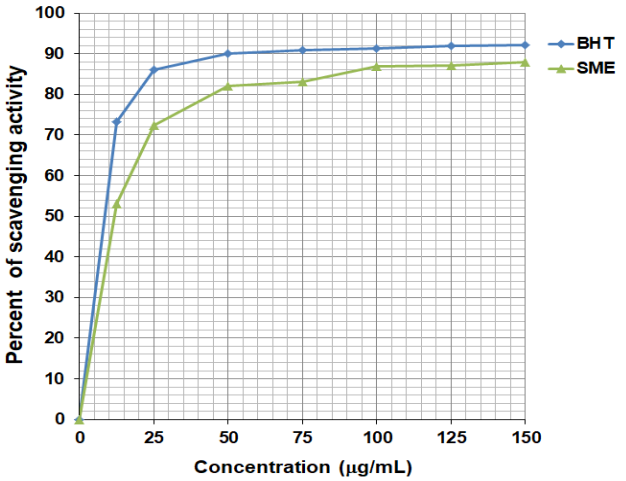


**Figure 2.** DPPH radical scavenging activity (%) of *Silybum marianum* seeds ethanolic extract (SME) and standard (Butylated hydroxytoluene, BHT). Each value represents the mean value of three replicates.

**Table 3. $IC_{50}$ (DPPH) of *Silybum marianum* extract (SME) and standard (Butylated hydroxytoluene, BHT).**

| Name of sample | BHT | SME |
|---|---|---|
| $IC_{50}$ (µg/mL) | 8.21± 0.18 [b] | 11.61± 0.70[a] |

* Each value represents the mean value of three replicates ±SD. Values with different superscript letters in the same raw are significantly did different at p≤0.05.

### 3.1.4. Inhibition of low density lipoprotein (LDL) oxidation

Dose-dependent inhibition of $CuSO_4$-induced LDL oxidation *in vitro* by *Silybum marianum* seeds ethanolic extract (SME) is shown in Figure 3. Such data indicated that the inhibitive action of the SME against CuSO4-induced LDL oxidation, as evidenced by decreased conjugated dienes formation in a dose-dependent style. A comparative study between SME and caffeic acid (CA) clarified that SME acted strong dramatically in protecting LDL against oxidation. The present data with the others proved that such effect could be attributed to the different bioactive compounds as antioxidants (silymarin, phenolics, flavonoids, tannins, α-tocopherol, carotenoids, chlorophyll and anthocyanin's etc.) contained in such SME [54,69,78,88,89]. In similar study, Aly *et al.*, [90] found that some plant parts extracts contained the same bioactive compounds including onion skin, tomato pomace and eggplant peels protect LDL against oxidation *in vitro*. Also, the same effect was observed when applied with pomegranate juice, which was attributed to the high levels of phenolic compounds and ascorbic acid contained the same effect when applied with pomegranate juice or pomegranate peel extract which was attributed to the high levels of phenolic compounds and ascorbic acid contained in the juice [91]. Several studies mentioned that the protecting of LDL against oxidation by phenolic compounds could be explained by increasing the levels of reduced glutathione (GSH) and glutathione reductase (GSH-Rd) in liver and lungs as well as increase in inhibition of NADPH-dependent lipid peroxidation [78,85,92,93]. Also, phenolic compounds exhibited a complex reaction with peroxyl radicals ($ROO^{\cdot}$) and inhibition of the LDL oxidation [94,95]. With this context, several authors found that the ‘‘oxidative modification of lipoproteins’’ hypothesis proposes that LDL oxidation plays a key role in early atherosclerosis [91,96,97]. Such action, oxidized LDL is atherogenic, could be attributed to its cytotoxic effects toward arterial cells and stimulates the monocytes to be adhesive to the endothelium which bring to the evolution of atheromatous plaques [98]. Thus, results of the present study with the others proved that the SME could be successfully used as a promising agent in the forbidding of atherosclerosis through inhibiting LDL oxidation process.

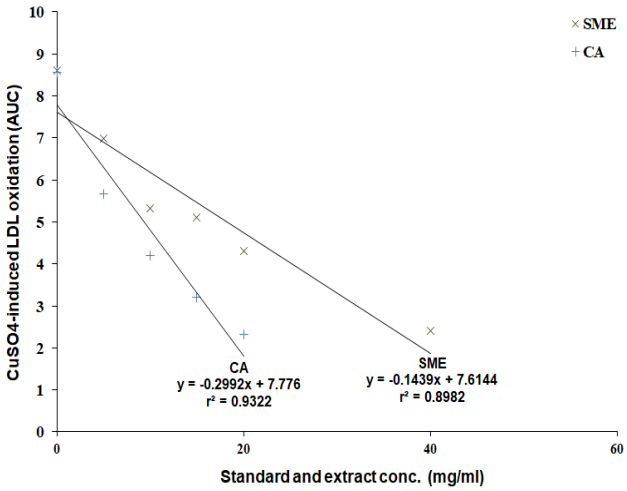


**Figure 3.** Dose-dependent inhibition of CuSO4-induced LDL oxidation *in vitro* by *Silybum marianum* seeds ethanolic extract (SME) and standard caffeic acid (CA). Result is expressed as the area under the curve (AUC).

## 3.2. Biological studies

### 3.2.1. The effect of intervention with *Silybum marianum* extract (SME) on body weight gain (BWG) of obese rats

Data in Table 4 and Figure 4 were shown the effect of SME on body weight gain (BWG) of obese rats. Such data indicated that feeding of rats on DIO (model control) leads to increase the body weight than the normal control group. Rats of the normal group recorded 68.02% of baseline for the body weight while model control group was 119.85% of baseline at the end of the experiment period (8 weeks). Intervention with SME (200, 400, 600 and 800 mg/kg bw/day) in feeding rats protocol for 8 weeks led to significantly ($p \leq 0.05$) decrease on the body weight of the obese rats (model control) which recorded 96.13, 88.36, 77.11 and 76.30% of the baseline, respectively. The rate of decreasing in BWG of the obese rats was exhibited a

dose- dependent increase with the SME intervention. Such data are in accordance with several studies reviewed by Tajmohammadi [37].All of these studies found that the positive effects of SME regarding the obese/ model control group could be attributed to its high level content of different categories of bioactive compounds principally silymarin, a complex mixture of polyphenolic molecules, including seven closely related flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin) and one flavonoid (taxifolin). Another study of Sayin et al.,[35] found that silymarin induced body weight decreased significantly in rats after 11 weeks of feeding on high fat diet-induced obesity. In contrast, Guo et al., [99] found that silymarin did not effect on lean body weight but significantly reduced fat accumulation. Also, Yao et al., [100] reported that silybin increased gene and protein expreussions of adiponectin that can enhance β-oxidation of free fatty acids and reduce de novo free fatty acid production within the hepatocytes which forbidding lipid accumulation. Furthermore, Ka et al.,[17] mentioned that_silybin reduced lipogenesis in mature adipocytes and inhibited differentiation in preadipocytes through many mechanisms including regulating insig-1 and -2 can reduce adipogenesis associated genes expression such as CAAT or enhancer binding protein-α, fatty acid synthase, SREBP1c, adipocyte specific lipid binding protein, PPARγ and lipoprotein lipase, a preadipocyte marker gene. On the other side, the effect of bioactive compounds other than silymarin which found in SME (phenolics, carotenoids, flavonoids, tannins etc.) in the control of obesity is confirmed by several studies [10,80,101-105]. Such bioactive compounds have been shown to impact gene expression and cell (including adipocyte) function through one or several of the following mechanisms, interacting with several transcription factors of the nuclear receptor superfamily, interfering with the activity of other transcription factors, modulating signaling pathways which are associated with inflammatory and oxidative stress responses, and extra-genomic actions such scavenging of reactive species. After all the studies that have been mentioned in addition to the results of the current study the antiobesity effects of SME could be attributed to several mechanisms including antioxidant/scavenging and anti-inflammatory activities, inhibition of the LDL oxidation, reduced fat accumulation, decreased leptin and resistin levels, increased lipolysis and adiponectin, inhibited differentiation of adipocytes, and reduced adipogenesis.

**Table 4. The effect of intervention with *Silybum marianum* extract (SME) on body weight gain (g) of obese rats***

| Groups | Feeding period (weeks) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Normal control | 158.34 | 164.44 | 171.83 | 185.68 | 206.29 | 225.31 | 243.65 | 254.66 | 266.05 $^{d}$ |
| Model control | 158.34 | 170.54 | 202.81 | 220.67 | 257.04 | 291.54 | 316.84 | 338.19 | 348.11 $^{a}$ |
| SME intervention (200 mg/kg bw/day) | 158.34 | 169.43 | 192.56 | 210.65 | 234.22 | 261.67 | 280.32 | 294.50 | 310.56 $^{b}$ |
| SME intervention (400 mg/kg bw/day) | 158.34 | 169.03 | 189.31 | 206.00 | 225.10 | 254.31 | 272.19 | 282.48 | 298.25 $^{b}$ |
| SME intervention (600 mg/kg bw/day) | 158.34 | 168.43 | 187.78 | 202.11 | 212.07 | 239.65 | 257.97 | 268.09 | 280.43 $^{c}$ |
| SME intervention (800 mg/kg bw/day) | 158.34 | 167.09 | 183.34 | 195.13 | 208.64 | 236.98 | 251.43 | 264.39 | 279.16 $^{cd}$ |

* Each value represents mean results of six animals. Means with different superscript letters on the same column indicate significant difference ($P \leq 0.05$). Normal control: healthy rats without intervention; Model control: HFD induced obesity rats without intervention; SME intervention: HFD induced obesity rats with SME intervention. bw, body weight.

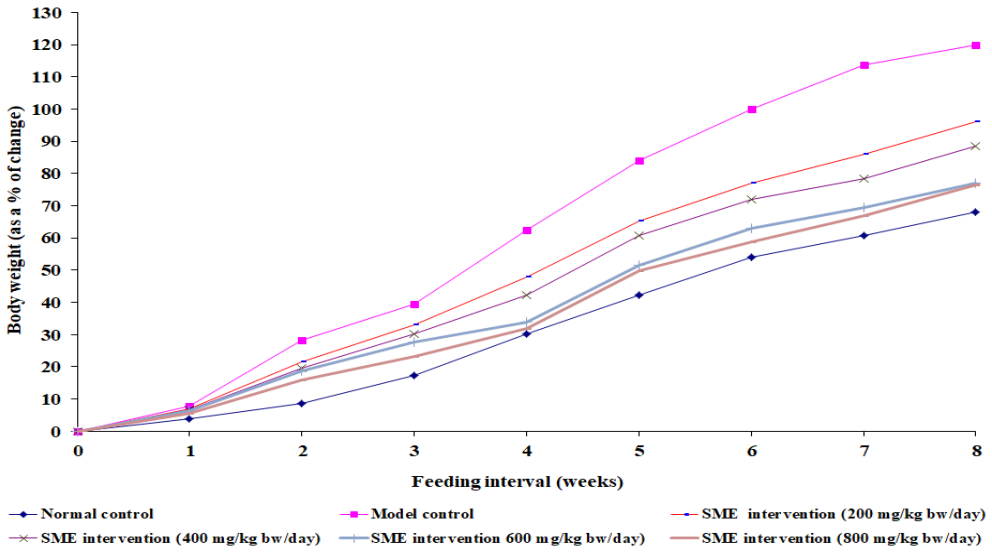


**Figure 4.** The effect of intervention with *Silybum marianum* extract (SME) on body weight gain (as a percent of change from control) of obese rats. Each value represents mean results of six animals. Normal control: healthy rats without intervention; Model control: HFD induced obesity rats without intervention; SME intervention: HFD induced obesity rats with SME intervention. bw, body weight.)

### 3.2.2. Effect of intervention with *Silybum marianum* extract (SME) on liver function of obese rats

The effect of SME intervention on liver functions of obese rats was shown in Table 5 and Figures (5 and 6). From such data it could be noticed that DIO caused a significant ($p \leq 0.05$) increased in AST, ALT and ALP with 51.63, 51.65 and 37.25% compared to normal control group, respectively. Intervention with SME (200, 400, 600 and 800 mg/kg bw/day) in feeding rats protocol for 8 weeks led to significantly ($p \leq 0.05$) decrease the levels of those enzymes activities which recorded 34.13, 19.75, 19.15 and 8.98% (for AST), 43.52, 26.23, 18.61 and 14.41% (for ALT), and 33.80, 26.61, 18.79 and 15.51% (for ALP) compared to the normal controls, respectively. The rate of decreasing in serum liver enzymes activities were exhibited a dose- dependent increase with the SME intervention. Such data are in accordance with several studies those reviewed by Abenavoli et al., [29]. In general, aminotransferases (AST and ALT) plus ALP are normally intracellular enzymes. Therefore, the presence of elevated levels of these enzymes in the plasma indicates damage to cells rich out. For example, liver and pancreas diseases process can cause cell lysis resulting to release of intracellular enzymes into the blood [106-109]. In the present study, SME bioactive constituents play several vital roles in terms of their hepatoprotective effects. With the same context, Krecman et al., [110] found that silymarin exerts hepatoprotective effect through its antioxidant and increasing intracellular and liver glutathione (GSH) level and scavenging free radicals. Administration of 26.25 mg/kg/day of silybin for 6 weeks in rats showed significantly protect against high fat-induced fatty liver by different mechanisms including effective in stabilizing mitochondrial membrane fluidity and decreasing oxidative stress. Also, Di Sario et al., [111] revealed that silybin exerted hepatoprotective, anti-inflammatory, and antifibrotic effects in rats administrated with dimethylnitrosamine. Furthermore, silybin can act against NASH (nonalcoholic steatohepatitis)-induced liver damage by insulin reduction, antioxidant, and hepatoprotective effect [112]. Recently, Badawy,[78] and Elhassaneen et al., [108] found that the consumption of *Silybum marianum* seed powder on carbon tetrachloride hepatotoxic rats lead to significantly improve in liver functions and histology through its antioxidant mechanism. In another study, high fat diet mice treatment with silymarin exhibited antidiabetic and liver protective effects through decreasing reactive oxygen species (ROS), protein oxidation, and lipid peroxidation as well as reducing the expression of hepatic NADPH oxidase and activation of NF-κB, enhanced the expression of hepatic NO, and modulating antioxidant activity of enzymes such as SOD, CAT, and GSH-Px in plasma and liver [113]. On the other side, several previous studies indicated that different parts rich in polyphenolic compounds rather than *Silybum marianum* could be lowered liver serum enzymes through many suggested effects including block the hepatocellular uptake of bile acids and improved the antioxidant capacity of the liver, diminished the bilirubin concentration reduce the damage of hepatocytes, and scavenged of the reactive oxygen species (ROS) [10, 38, 107].

**Table 5. Effect of intervention with *Silybum marianum* extract (SME) on liver function of obese rats**

| Group | AST (IU/L) | ALT (IU/L) | ALP (U/L) |
|---|---|---|---|
| Normal control | 41.91± 3.90 $^{d}$ | 25.09 ± 1.99 $^{c}$ | 96.16 ± 8.99 $^{c}$ |
| Model control | 63.54 ± 5.10 $^{a}$ | 38.05 ± 4.21 $^{a}$ | 131.98 ± 9.29 $^{a}$ |
| SME intervention (200 mg/kg bw/day) | 56.21± 2.76 $^{ab}$ | 36.01 ± 3.78 $^{a}$ | 128.66 ± 8.66 $^{a}$ |
| SME intervention (400 mg/kg bw/day) | 50.18 ± 6.21 $^{b}$ | 31.67 ± 1.87 $^{bc}$ | 121.75 ± 9.57 $^{ab}$ |
| SME intervention (600 mg/kg bw/day) | 49.93 ± 4.31 $^{b}$ | 29.76 ± 5.43 $^{c}$ | 114.23 ± 15.25 $^{b}$ |
| SME intervention (800 mg/kg bw/day) | 45.67 ± 7.21 $^{bc}$ | 28.71 ± 3.91 $^{c}$ | 111.08 ± 12.19 $^{b}$ |

Each value represents mean ±SD (n=6). Means on the same column with different superscript letters explain significant difference at P≤ 0.05. Normal control, healthy rats without intervention; Model control, HFD (high fat diet) induced obesity rats without intervention; SME intervention, HFD induced obesity rats with SME intervention; bw, body weight; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

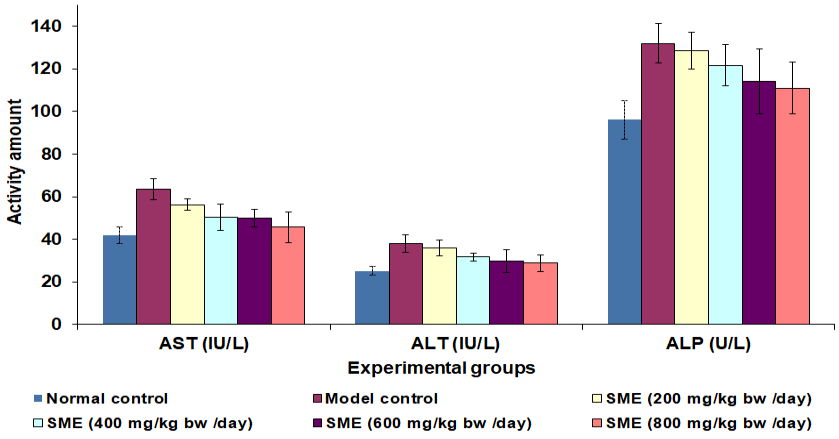


**Figure 5.** Effect of intervention with *Silybum marianum* extract (SME) on liver function of obese rats (Each value represents mean ±SD (n=6). Means on the same column with different superscript letters explain significant difference at P≤ 0.05. Normal control, healthy rats without intervention; Model control, HFD (high fat diet) induced obesity rats without intervention; SME intervention, HFD induced obesity rats with SME intervention; bw, body weight; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.)

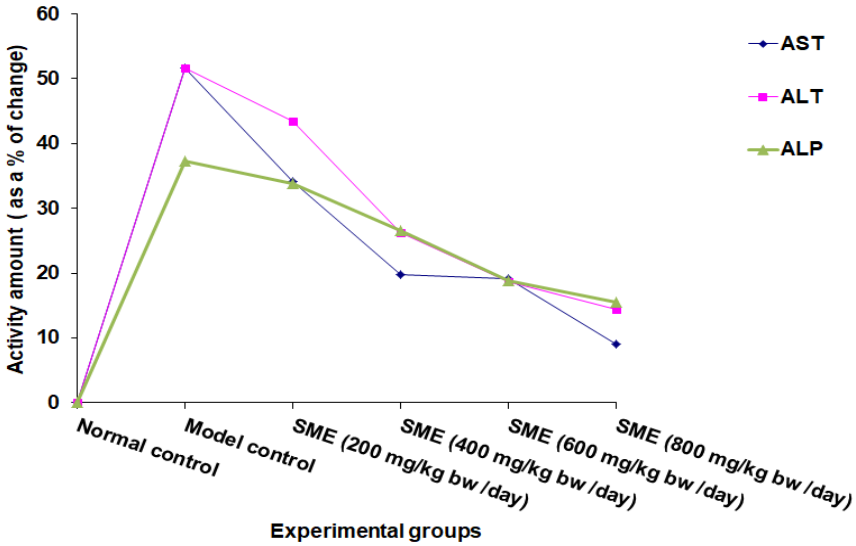


**Figure 6.** Effect of intervention with *Silybum marianum* extract (SME) on liver function of obese rats (Each value represents mean reading of six animals. Normal control, healthy rats without intervention; Model control, HFD (high fat diet) induced obesity rats without intervention; SME intervention, HFD induced obesity rats with SME intervention; bw, body weight; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.)

### 3.2.3. Effect of intervention with *Silybum marianum* extract (SME) on serum lipid profile of obese rats

Data in Table 6 and Figures (7 and 8) were shown the effect of SME on blood lipid profile of obese rats. From such data it could be noticed that treatment of rats with DOI caused a significant ($p \leq 0.01$) increased in serum TGs, TC, LDL-c and VLDL-c by the ratio 232.89, 69.09, 163.41 and 232.89% compared to normal controls. Intervention with SME by 200, 400, 600 and 800 mg/kg bw/day in feeding rats protocol for 8 weeks led to significantly ($p \leq 0.05$) decrease the levels of TGs, TC, LDL-c and VLDL-c which recorded 218.42, 209.21, 202.63 and 122.37%; 65.62, 57.41, 41.64 and 25.55%; 155.20, 126.57, 91.27 and 56.74%, and 218.42, 209.21, 202.63 and 122.37% compared to the normal controls, respectively. The opposite direction was observed for HDL-c level. The rate of decreasing in TGs, TC, LDL-c and VLDL-c and increasing in HDL-c levels were exhibited a dose-dependent increase with SME intervention. Such data indicated that obesity associated with hyperlipidemic and hypercholesterolemic i.e. increased the serum bad lipid particles (TGs, TC, LDL-c and VLDL-c) and decrease the good one (HDL-c) which significantly improved by SME intervention. Previous studies recorded the same behavior with different pant parts other than *Silybum marianum* seeds [12,14,16,23] In general, high serum lipid and lipoprotein levels, particularly LDL and VLDL, elevate the risk of CVD, fatty liver, carcinogenesis, peripheral vascular disease, and atherosclerosis in human [114]. There are many new synthetic oral antihyperlipidemic and antihypercholesterolemic drugs, but they have adverse side effects such as myopathy, increase in hepatic aminotransferases, and rhabdomyolysis condition [115]. Data of the present study showed the effectiveness of SME in improving the blood lipid profile resulting from feeding of DIO without any side effects. Such data are in agree with Sobolova et al., [116] who observed that administration of silymarin and polyphenolic fraction of silymarin significantly decreased cholesterol absorption, the plasma level of cholesterol, liver content of VLDL-c, and TGs. Reducing VLDL-c level was due to several factors including reduction of VLDL-c formation and secretion from the liver, reduction of VLDL-c secretion in the intestine, and inhibition of intestinal cholesterol absorption. Also, Radjabian and Fallah Huseini, [117] found that silymarins decreased significantly levels of TC, LDL-c, and TGs, inhibited atherosclerotic plaque formation, and increased significantly HDL-c in rabbit serum. Furthermore, Gobalakrishnan et al., [118] noticed that oral administration of silybin significantly decreased TC, TGs, LDL-c, VLDL-c and increased HDL-c levels at both serum and liver in high cholesterol diet fed rats. After all the studies that have been mentioned in addition to the results of the current study the antihyperlipidemic and antihypercholesterolemic effects of SME could be attributed to several mechanisms including stimulating the production of hepatic LDL-c receptors that raised clearance of plasma LDL-c and VLDL-c, increasing the endogenous cholesterol conversion to bile acid, inhibition of acyl-CoA such as cholesterol acyltransferase as a key pathway of lipid metabolism which could be effective in reduction of intestinal cholesterol absorption, and the polymeric structure of silymarin fractions may bind to cholesterol and bile acids.

**Table 6. Effect of intervention with *Silybum marianum* extract (SME) on serum lipid profile of obese rats**

| Group | TG (mmol/L) | TC (mmol/L) | HDL-c (mmol/l) | LDL-c (mmol/l) | VLDL-c (mmol/l) |
|---|---|---|---|---|---|
| Normal control | 0.76 ± 0.10 $^{d}$ | 3.17 ± 0.58 $^{c}$ | 1.46 ± 0.02 $^{a}$ | 1.56 ± 0.11 $^{c}$ | 0.15 ± 0.08 $^{c}$ |
| Model control | 2.53 ± 0.24$^{a}$ | 5.36 ± 0.54 $^{a}$ | 0.75 ± 0.41 $^{c}$ | 4.10 ± 0.58 $^{a}$ | 0.51 ± 0.10 $^{a}$ |
| SME intervention (200 mg/kg bw/day) | 2.42 ± 0.18 $^{a}$ | 5.25 ± 0.46 $^{a}$ | 0.79 ± 0.27 $^{bc}$ | 3.98 ± 0.69 $^{a}$ | 0.48 ± 0.08 $^{a}$ |
| SME intervention (400 mg/kg bw/day) | 2.35 ± 0.09 $^{ab}$ | 4.99 ± 0.13 $^{ab}$ | 0.99 ± 0.34 $^{b}$ | 3.53 ± 0.44 $^{ab}$ | 0.47 ± 0.09 $^{a}$ |
| SME intervention (600 mg/kg bw/day) | 2.30 ± 0.27 $^{b}$ | 4.49 ± 0.11 $^{b}$ | 1.05 ± 0.39 $^{ab}$ | 2.98 ± 0.3 $^{b}$ | 0.46 ± 0.10 $^{a}$ |
| SME intervention (800 mg/kg bw/day) | 1.69 ± 0.25 $^{c}$ | 3.98 ± 0.32 $^{bc}$ | 1.20 ± 0.19 $^{a}$ | 2.44 ± 0.19 $^{bc}$ | 0.34 ± 0.07 $^{b}$ |

Each value represents mean ±SD (n=6). Means on the same column with different superscript letters explain significant difference at P≤ 0.05. Normal control, healthy rats without intervention; Model control, HFD (high fat diet) induced obesity rats without intervention; SME intervention, HFD induced obesity rats with SME intervention; bw, body weight; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

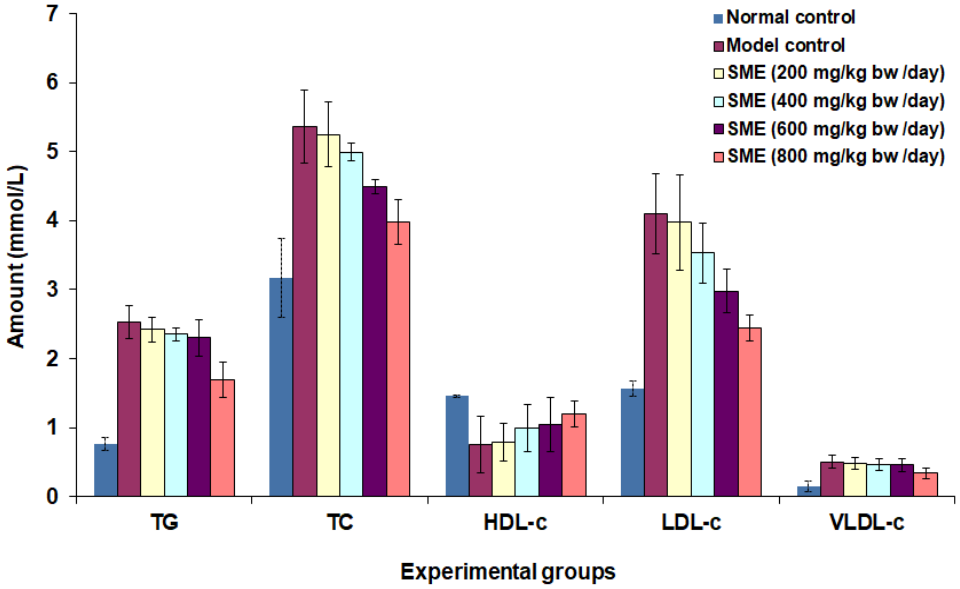


**Figure 7.** Effect of intervention with *Silybum marianum* extract (SME) on serum lipid profile of obese rats (Each value represents mean ±SD (n=6). Means on the same column with different superscript letters explain significant difference at P≤ 0.05. Normal control, healthy rats without intervention; Model control, HFD (high fat diet) induced obesity rats without intervention; SME intervention, HFD induced obesity rats with SME intervention; bw, body weight; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.)

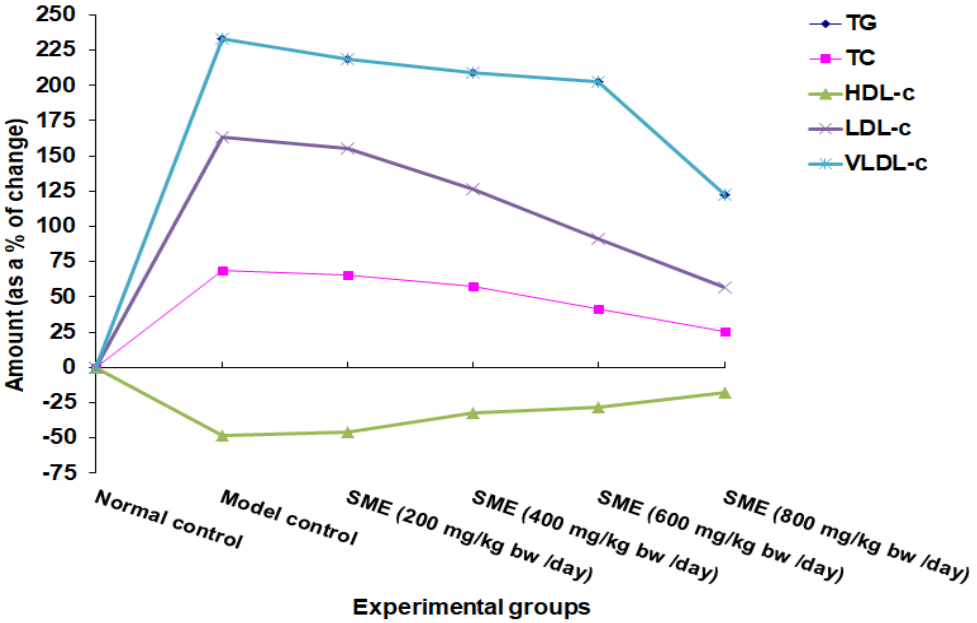


**Figure 8.** Effect of intervention with *Silybum marianum* extract (SME) on serum lipid profile of obese (Each value represents mean reading of six animals. Normal control, healthy rats without intervention; Model control, HFD (high fat diet) induced obesity rats without intervention; SME intervention, HFD induced obesity rats with SME intervention; bw, body weight; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.)

## 3.3. Histopathological studies

### 3.3.1. Effect of intervention with *Silybum marianum* extract (SME) on liver and adipose tissue histopathological examination of obese rats

#### 3.3.1.1. Liver

Effect of intervention with *Silybum marianum* extract (SME) on liver histopathological examination of obese rats was shown in Figure 9. Microscopically, liver of rats from group 1 revealed the normal histological architecture of hepatic lobule (Photo 1). In contrariwise, liver of rats from group 2 showed hepatocellular vacuolar degeneration, focal hepatic hemorrhage (photo 2), and focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration (Photo 3). Meanwhile, liver of rats from group 3 exhibited hepatocellular vacuolar degeneration and hepatic hemorrhage (Photo 4). On the other hand, liver of rats from group 4 revealed slight congestion of hepatic sinusoids (Photo 5) and slight vacuolization of some hepatocytes (Photo 6). Furthermore, liver of rats from group 5 exhibited vacuolization of some hepatocytes (Photo 7). Otherwise, examined sections from group 6 showed slight congestion of hepatic sinusoids (Photo 8).

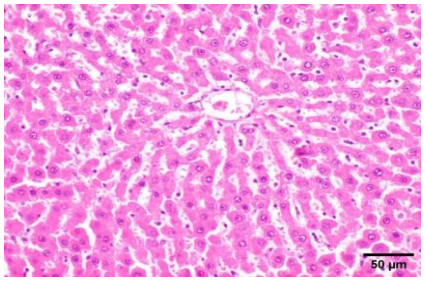


**Photo 1**. Photomicrograph of liver of rat from group 1 showing the normal histological architecture of hepatic lobule

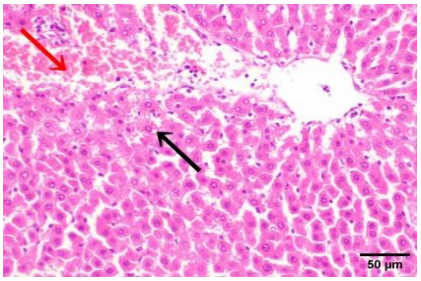


**Photo 2.** Photomicrograph of liver of rat from group 2 showing hepatocellular vacuolar degeneration (black arrow) and focal hepatic hemorrhage (red arrow)

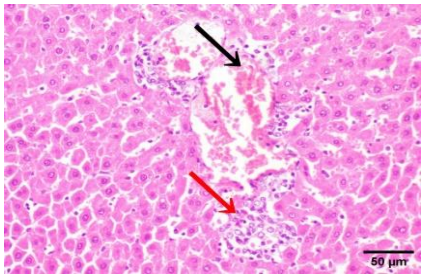


**Photo 3.** Photomicrograph of liver of rat from group 2 showing congestion of central vein (black arrow) and focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration (red arrow)

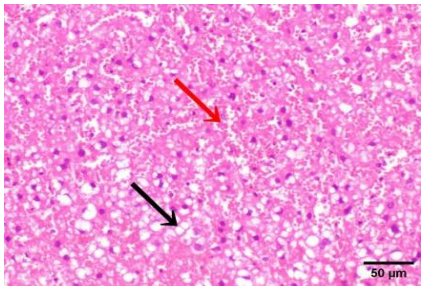


**Photo 4.** Photomicrograph of liver of rat from group 3 showing hepatocellular vacuolar degeneration (black arrow) and hepatic hemorrhage (red arrow)

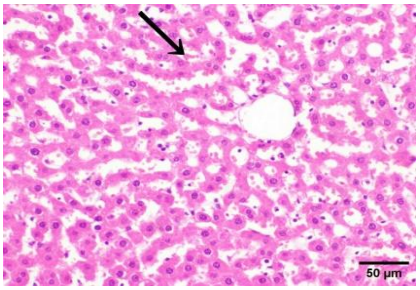


**Photo 5.** Photomicrograph of liver of rat from group 4 showing slight congestion of hepatic sinusoids (arrow)

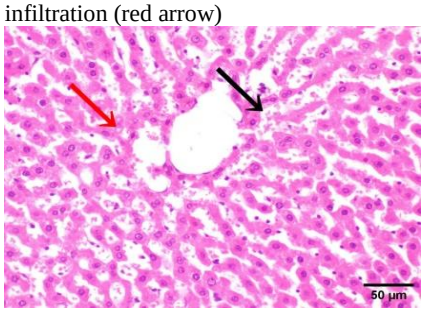


**Photo 6.** Photomicrograph of liver of rat from group 4 showing slight congestion of hepatic sinusoids (black arrow) and slight vacuolization of some hepatocytes (red arrow)

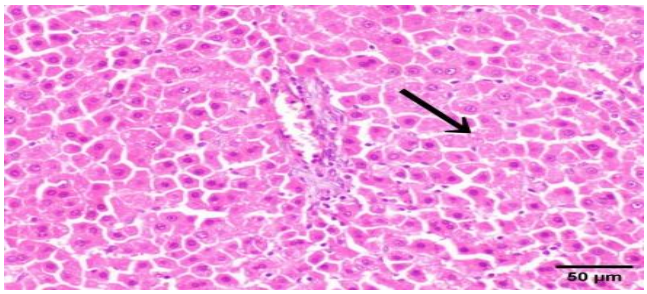


**Photo 7.** Photomicrograph of liver of rat from group 5 showing slight vacuolization of some hepatocytes (arrow)

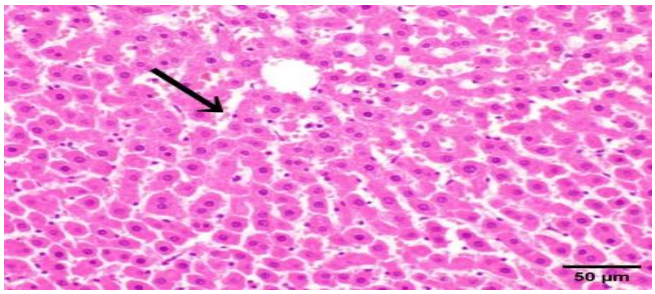


**Photo 8.** Photomicrograph of liver of rat from group 6 showing slight congestion of hepatic sinusoids (arrow

**Figure 9.** Effect of intervention with Silybum marianum extract (SME) on liver histopathological examination of obese rats. (H & E, scale bar 50 µm, X 200).

In similar study, Shaker et al., [119] showed that the histopathological examination for rat livers observes no alteration for rat control, while focal necrosis and diffuse kupffer cells proliferation in between the hepatocytes for $CCl_4$ treated group. Some improvements have been reported in the protective groups as dilatation in the hepatic sinusoids associated with inflammatory cells infiltration and diffuse kupffer cells proliferation in between the degenerated hepatocytesas the result of SME treatment. Also, Shimaa et al., [120] found that hidtological examination of the rats liver pretreated with the SME at dose 200 mg/kg b.wt. for 2 month decrease the

severity of histopathological changes induced by carbon tetrachloride group. Liver sections examination showed normal hepatocytes and sinusoids except in few sections showed focal area of hepatocellular necrosis replaced with mononuclear cells with vascular degeneration of the surrounding hepatocytes. The raising of the SME dose, 400 mg/kg b.wt., exhibited normal hepatocytes, hepatic cord, and sinusoids as well as kupffer cell activation. Furthermore, the applied milk thistle supplements have been proven effective in the prevention of histopathological changes caused by diets contaminated with mycotoxins, which include vacuolar hepatocyte degeneration (solitary cell death and infiltration of lympho- and histiocytes in the liver) [121].

#### 3.3.1.2. Adipose tissue

Effect of intervention with Silybum marianum extract (SME) on adipose tissue histopathological examination of obese rats was shown in Figure 10. Microscopically, adipose of rat from group 1 revealed normal unilocular adipocytes, polygonal in shape and having signet ring appearance (Photo 1). In contrast, adipose tissue of rats from group 2 showed histopathological alterations characterized large size unilocular adipocytes (Photo 2) and inflammatory cells infiltration (Photo 3). Meanwhile, some sections from group 3 showed focal hemorrhage (Photo 4). Otherwise, some sections from group 4 revealed few large size unilocular adipocytes and some small size adipocytes (Photo 5). Likewise, adipose tissue of rats from group 5 showed few large size unilocular adipocytes (Photo 6). Furthermore, most examined sections from group 6 exhibited apparent histologically normal unilocular adipocytes (Photo 7). Such observation are in accordance partially with thatreported by Alsaggar et al., [122] who showed fat accumulation in major fat white adipose tissues, WAT, (tissues (epididymal WAT and inguinal WAT), and the expansion of adipocytes were both suppressed by silibinin, main bioactive compound in SME, treatment. Suppressed fat accumulation and adipose tissue hypertrophy was investigated of white and brown adipose tissues (WAT and BAT).

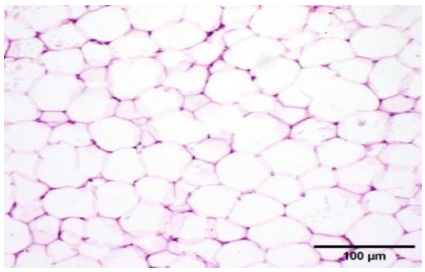


**Photo 1.** Photomicrograph of adipose tissue of rat from group 1 showing normal unilocular adipocytes, polygonal in shape and having signet ring appearance.

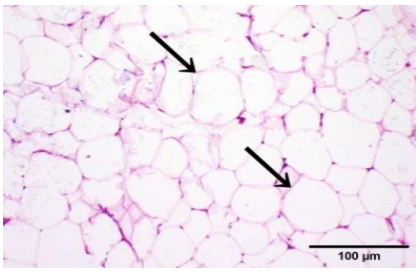


**Photo 2.** Photomicrograph of adipose tissue of rat from group 2 showing large size unilocular adipocytes (black arrow).

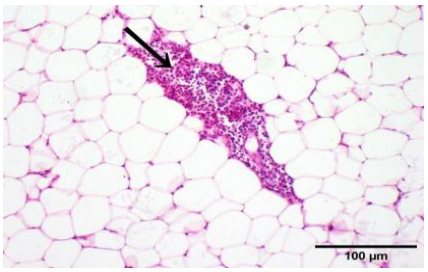


**Photo 3.** Photomicrograph of adipose tissue of rat from group 2 showing inflammatory cells infiltration (black arrow).

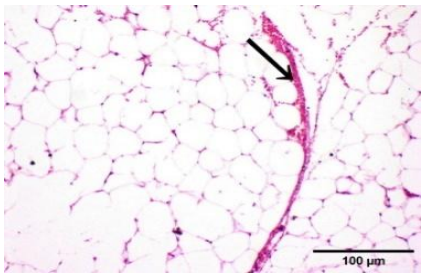


**Photo 4.** Photomicrograph of adipose tissue of rat from group 3 showing focal hemorrhage (black arrow).

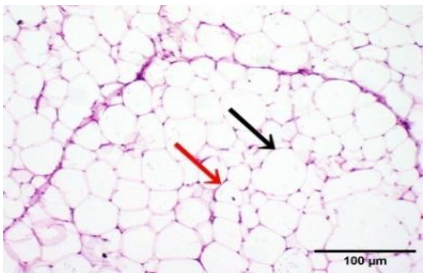


**Photo 5.** Photomicrograph of adipose tissue of rat from group 4 showing few large size unilocular adipocytes (black arrow) and some small size adipocytes (red arrow).

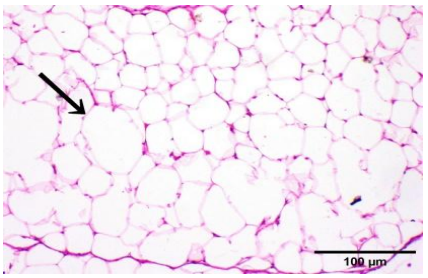


**Photo 6.** Photomicrograph of adipose tissue of rat from group 5 showing few large size unilocular adipocytes (black arrow).

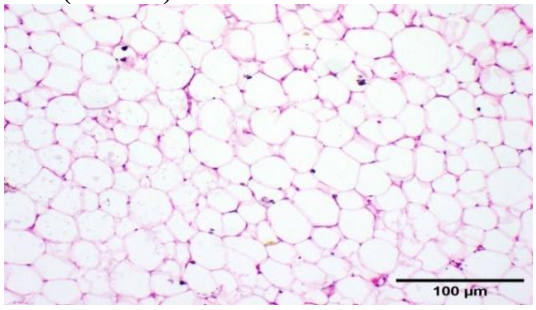


**Photo 7.** Photomicrograph of adipose tissue of rat from group 5 showing apparent histologically normal unilocular adipocytes

**Figure 10**. Effect of intervention with *Silybum marianum* extract (SME) on adipose tissue histopatho-logical examination of obese rats. (H & E, scale bar 100 µm, X 100)

# 4. Conclusion

Data of the present study supported our hypothesis that *Silybum marianum* ethanolic extract (SME) contains several categories of phytochemicals including silymarin, phenolics, flavonoids, tannins, α-tocopherol, chlorophyll, β-carotene, anthocyanin's with other compounds that are responsible for different biological activities. The biological activities studied here including scavenging activity of free radicals and inhibition of low density lipoprotein oxidation. Such important biological effects could play important roles in strategies to combat/treat of obesity and its complications. Anti-obesity effects of SME was probably attributed to several mechanisms such as it has beneficial effects for obesity-related reducing fat accumulation and body weight gains, protecting against liver damages, improving blood lipids components, positively manipulate of obesity-related histopathological changes and so on. Thus, data of the present study recommended like of that *Silybum marianum* extracts to be included in our daily diets, drinks, food supplementation and pharmacological formulae for the obese patients.

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# Conflicts of Interest

The authors declare no conflict of interest.

# References

[1] Nammi, S., Saisudha, K., Krishna, M. & Krishna, M. (2004): Obesity: An overview on its current perspectives and treatment options .*Nutrition Journal*, 3: 1-8

[2] Taylor, R.W., Keil, D., Gold, E.J., Williams, S.M. & Goulding, A.(1998). Body mass index, waist girth and waist-to-hip ratio as indexes of total and regional adiposity in women: evaluation using receiver operating characteristic curves. *Am J Clin Nutr*, 67:44-49.

[3] Caterson, I.D. (2009). Medical management of obesity and its complications. *Annals of the Academy of Medicine, Singapore*, 38(1), 22-27.

[4] Muñoz-Garach, A., Diaz-Perdigones, C. & Tinahones, F. J. (2016). Gut microbiota and type 2 diabetes mellitus. Microbiota y diabetes mellitus tipo 2. *Endocrinologia y* (10):560-568.

[5] Felisbino-Mendes, M., Cousin, E., Malta, D., Ísis, E., Antonio, L., Bruce, B., Maria, I., Diego, A., Scott, G., Ashkan, A. & Gustavo, V. (2020). The burden of non-communicable diseases attributable to high BMI in Brazil, 1990–2017: findings from the Global Burden of Disease Study. *Popul Health Metrics* 18 (Suppl 1): 1-13

[6] Mehrzad, R . (2020). The Global Impact of Obesity. In: Mehrzad R, editor. Obesity. Amsterdam*: Elsevier*. 55-72

[7] Ramasamy, A ., Laliberté, F., Aktavoukian, S.A., Lejeune, D., DerSarkissian, M., Cavanaugh, C., Smolarz, B.G., Ganguly, R. & Duh, M.S.(2019) Direct and Indirect Cost of Obesity Among the Privately Insured in the United States: A Focus on the Impact by Type of Industry. *J Occup Environ Med*, 61(11):877–886.

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

[9] Alexopoulos, A.S., Fayfman, M., Zhao, L., Weaver, J., Buehler, L., Smiley, D. & Umpierrez, G. E. (2016). Impact of obesity on hospital complications and mortality in hospitalized patients with hyperglycemia and diabetes. *BMJ Open Diabetes Research and Care*, 4(1): 1144 -1150.

[10] Elmaadawy, A., Rasha, M., Arafa. & Yousif, Elhassaneen. (2016). Oxidative Stress and antioxidant defense systems status in obese rats feeding some selected food processing by-products applied in bread. *Journal of Home Economics*, 26 (1): 55-91

[11] Mehram, E.B., Alaa O. Aboraya. & Yousif A. Elhassaneen (2021). Potential Effects of Food Processing Byproducts on Neurological and Immunological Disorders of Obese Rats. *Alexandria Science Exchange Journal*, 42 (2): 509-522.

[12] Shalaby, H. & Elhassaneen, Y. (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 Science Exchange Journal*, 42(2): 559-571. [DOI: 10.21608/asejaiqjsae.2021.181848]

[13] Elhassaneen, Y., Mekawy, S., Khder, S. & Salman, M. (2019). Effect of Some Plant Parts Powder on Obesity Complications of Obese Rats. *Journal of Home Economics*, 29 (1): 83-106. [DOI: 10.21608/mkas.2017.166177].

[14] Elhassaneen, Yousif., Mohamed, El-Dashlouty. & Nilly El-Gamal (2020a). Effects of brown algae (Sargassum subrepandum) consumption on obesity induced changes in oxidative stress and bone indices. *Journal of Home Economics*, 30 (4): 687-708

[15] Elhassaneen, Y., Sayed, Ahmed. S., Elwasef, S. & Fayez, S. (2022 a). Effect of brown algae ethanolic extracts consumption on obesity complications induced by high-fat diets in rats. *Port Saied Specific Research Journal (PSSRJ)*,15 (1).

[16] Elhassaneen, Y., Hassab El-Nabi, S.E., Bayomi, A. I., & ElKabary, A.R (2022 b). Potential of watermelon (*citrullis lanatus*) peel extract in attenuating benzo[*a*]pyrene exposure-induced molecular damage in liver cells in vitro. *Journal of Biotechnology Research*, 8(3): 32-45.

[17] Ka, S.O., Kim, K.A., Kwon, K.B., Park, J.W. & Park, B.H. (2009). Silibinin attenuates adipogenesis in 3T3-L1 preadipocytes through a potential upregulation of the insig pathway. *International Journal of Molecular Medicine*, 23: 633- 637

[18] Grundy, S.M. (2004): Obesity, metabolic syndrome, and cardiovascular disease. J. Clin. Endocrinol. Metab. 89 (6): 2595–600.

[19] Elhassaneen, Y., Khairy, S. & Abd Elhamied, D. (2020-b). Dietary Pattern and Quality of Life among Obese Children. *Journal of Home Economics*,30 (1): 125-139.

[20] Cheng, K.C., Asakawa, A., Li, Y.X., Chung, H. H., Amitani, H., Ueki, T. & Inui, A. (2014). Silymarin induces insulin resistance through an increase of phosphatase and tensin homolog in Wistar rats. *PLoS One*, 9(1).

[21] Jandacek, R. J. & Woods, S. C. (2004). Pharmaceutical approaches to the treatment of obesity. *Drug Discov Today*, 9: 874-880.

[22] Elhassaneen, Y., El-Kholie, E., Zaki, A. & Yassen, H. (2018). Potential Effects of Cauliflower Leaves Powder on obese rats. Proceeding of the Annual Conference (13th Arab; 10th International), 11-12 April, Faculty of Specific Education, Mansoura University, " *Higher Education in Egypt and the Arab World in the Light of Sustainable Development Strategies", Mansoura, Egypt*

[23] Elhassaneen, Y., Nasef, A. and Abo-Khazima, A. (2020-c). Effect of coconut fruits -and their milk on biological and biochemical changes of hypercholesterolemic rats. *Journal of Home Economics*, 30 (1): 85-106. [DOI: 10.21608/mkas.2020.157712].

[24] Elhassaneen, Y., Ragab, S. & Essa, E. (2020-d). Chemical and nutritional studies on extracts of food processing by-products and their effects on obesity complications in rats. *Journal of Home Economics*,30 (2): 1-26. [DOI: 10.21608/mkas.2020.156506]

[25] Abenavoli, L., Capasso, R., Milic, N. & Capasso, F. (2010). Milk thistle in liver diseases: past, present, future. 24(10):1423-1432.

[26] Bijak, M. (2017). Silybin, a Major Bioactive Component of Milk Thistle (*Silybum marianum* L. Gaernt.)-Chemistry, Bioavailability, and Metabolism. *Molecules*. 10; 22(11):1942. doi: 10.3390/molecules22111942.

[27] Abd Elalal, N.S., Elsemelawy, S.A. & Elhassaneen, Y.A. (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 Journal of Healthcare and Medical Sciences*, 8(3): 19-34. doi.org/10.32861/ijhms.83.19.34.

[28] Flora, K., Hahn, M., Rosen, H. & Benner, K. (1998). Milk thistle (Silybum marianum) for the therapy of liver disease. *The American Journal of Gas- troenterology*, 93: 139-143.

[29] Abenavoli, L., Izzo, A.A., Milić, N., Cicala, C., Santini, A. & Capasso, R. (2018, November 1). Milk thistle (*Silybum marianum*): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. *Phytotherapy Research*, 32: 2202–2213.

[30] Kroll, D. J., Shaw, H.S. & Oberlies, N. H. (2007). "Milk Thistle Nomenclature: Why It Matters in Cancer Research and Pharmacokinetic Studies". *Integrative Cancer Therapies*, 6 (2): 110–9.

[31] Hogan, Fawn, S., Krishnegowda, Naveen, K., Mikhailova, Margarita, Kahlenberg. & Morton, S. (2007). "Flavonoid, Silibinin, Inhibits Proliferation and Promotes Cell-Cycle Arrest of Human Colon Cancer". *Journal of Surgical Research*. 143 (1): 58–65.

[32] Greenlee, H., Abascal, K., Yarnell, E. & Ladas, E. (2007). "Clinical Applications of Silybum marianum in Oncology". Integrative Cancer Therapies. 6 (2): 158–65.

[33] Soto, C., Mena, R., Luna, J., Cerbón, M., Larrieta, E., Vital, P. & Lara, A. (2004). Silymarin induces recovery of pancreatic function after alloxan damage in rats. *Life Sciences*, 75:2167- 2180.

[34] Poruba, M., Matušková, Z., Kazdová, L., Oliyarnyk, O., Malínská, H., Tozzi di Angelo, I. & Vecera, R. (2016). Positive effects of different drug forms of silybin in the treatment of the metabolic syndrome. *Physiological Research*, 64:507– S512.

[35] Sayin, F.K., Buyukbas, S., Basarali, M.K., Alp, H., Toy, H. & Ugurcu, V. (2016). Effects of Silybum marianum extract on high-fat diet-induced metabolic disorders in rats. *Polish J Food Nutr Sci*, 66:43– 49.

[36] Famouri, F., Salehi, M.M., Rostampour, N., Hashemi, E. & Shahsanaee, A. (2017). The effect of silymarin on the non-alcoholic fatty liver disease of children. *J Herbmed Pharmacol*, 6:16-20.

[37] Tajmohammadi, A., Bibi, M. & Hossein, H. (2018). Silybum marianum (milk thistle) and its main constituent, silymarin, as a potential therapeutic plant in metabolic syndrome: A review. *Phytotheray research*, 32 (10): 1933-1949

[38] Elhassaneen, Y., Abd El-Rahman, A. & El-Samouny, S. (2021-a). Potential Protective Effects of Cauliflower Leaves and Prickly Pear Fruits Skin on Liver Disorders Induced by Carbon Tetrachloride in Rats. *Journal of Home Economics*, 32 (1):19-42.

[39] Andrew, R. & Izzo, A.A. (2017). Principles of pharmacological research of nutraceuticals. *British Journal of Pharmacology*, 17: 1177– 1194.

[40] Oludemi, T., Sandrina, A., Ricardo, C., Maria, J., Lillian, B., Ana, M., Gonz, A., Maria, F. & Isabel, C. (2017). The potential of Ganoderma lucidum extracts as bioactive ingredients in topical formulations, beyond its nutritional benefits. *Food and Chemical Toxicology*, 108: 139-147

[41] Singleton, V. & Rossi, J. (1965) .Colorimetry of Total Phenolic Compounds with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16: 144-158.

[42] Wolfe, K., Wu, X. & Liu, RH. (2003). Antioxidant activity of apple peels. *J Agric Food Chem*, 51:609–614.

[43] Zhishen, J., Mengcheng, T. & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chemistry*, 64(4):555-559.

[44] Sukwattanasinit, T., Burana-Osot, J. & Sotanaphun, U.(2007) Spectrophotometric method for quantitative determination of total anthocyanins and quality characteristics of roselle (Hibiscus sabdariffa). *Planta Med.*73(14):1517-22

[45] Van-Burden, T.P. & Robinson, W.C. (1981). Fonnation of complexes between protein and tannic acid. *Journal of Agricultural and Food Chemistry*,1:77-88.

[46] Rajasekaran, A., Kumar, M., Krishnamoorthy, G. & Jayakar, B. (1997). Spectrophotometric determination of silymarin. *Indian J. Pharm*, 59(5): 230-231.

[47] Zilha, A., Lejla, G., Jasmina, H. & Senad, M. (2016). Spectrophotometric determination of total chlorophyll content in fresh vegetables. Works of the Faculty of Agriculture and Food Sciences, University of Sarajevo, 66(1): 104-107.

[48] Biswas, A.K., Sahoo, J. & Chatli, M,K. (2011). A simple UV-Vis spectrophotometric method for determination of β-carotene content in raw carrot, sweet potato and supplemented chicken meat nuggets. LWT .*Food Science and Technology*, 44 (8): 1809-1813.

[49] Tütem, E., Apak, R., Günaydı, E. & Sözgen, K.(1997) Spectrophotometric determination of vitamin E (alpha-tocopherol) using copper(II)-neocuproine reagent. *Talanta*, 44(2):249-55.

[50] Desmarchelier, C., Bermudez, M.J.N., Coussio, J., Ciccia, G. & Boveris, A. (1997). Antioxidant and prooxidant activities in aqueous extract of Argentine plants. Int. *J. Pharmacogn*. 35:116-120.

[51] Princen, H.M.G., Van-Poppel, G., Vogelezang, C., Buytenhek, R. & Kok, F.J. (1992). Supplementation with vitamin E but not bcarotene in vivo protects low-density lipoprotein from lipid peroxidation in vitro. *Arteriosclerosis and Thrombosis*, 12: 554-562.

[52] Reeves, P., Nielsen, F. & Fahey, G. (1993). "AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet". *Journal of Nutrition*, 123(11): 1939-1951.

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

[54] Elhassaneen, Y., Hanaa Badran., Abeer Abd EL-Rahman. and Naglaa Badawy (2021). Potential Effect of Milk Thistle on Liver Disorders Induced by Carbon Tetrachloride. *Journal of Home Economics*, 31 (1): 83-93.

[55] Drury, R.A.B. & Wallington, E. A. (1980). Carleton's histological technique 5th ed. *New York: Churchill Livingstone.*

[56] Yound, D. S. (1975). Determination of GOT. *Clin. Chem*, 22 (5): 21-27.

[57] Tietz, N.W. (1976): Fundamentals of Clinical Chemistry. Philadelphia. B.W. *Standers*, 243.

[58] Held, P. (2009). Determination of Insulin Levels in Human Serum. BioTek Instruments, Inc., Winooski, Vermont, USA

[59] Fossati, P. & Prenape, L. (1982): Serum triglycerides deter-mined colorimeterically with enzyme that produce hydrogen peroxide. *Clin. Chem*, 28: 2077-2080.

[60] Richmond, W. (1973). "Preparation and Properties of a Cholesterol Oxidase from *Nocardia sp*. and its Application to the Enzy-matic Assay of Total Cholesterol in Serum. *Clinical Chemistry*, 19:1350-1356.

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

[62] Fniedewald, W.T., Leve, R.L. & Fredrickson, D.S. (1972): Estimation of concentration of low density lipo protein separated by three different *Clin.Chem*, 18:499-502.

[63] Ellman, G.L. (1959): Tissue sulphydryl groups. Archives of Biochemistry and Biophysics 82: 70-77 .

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

[65] Erel, O. A. (2005). "New automated colorimetric method for measuring total oxidant status". *Clin Biochem*, 38:1103–11.

[66] Fathi-Achachlouei, B. & Azadmard-Damirchi, S. (2009). Milk Thistle Seed Oil Constituents from Different Varieties Grown in Iran. *Journal of the American Oil Chemists' Society,* 86(7):643–649.

[67] Harrabia, S., Romdhaneb, H., Daassab, M. & Fellaha, H. (2015). Fatty acid and triacylglycerol compositions of milk thistle seeds growing wild in Tunisia (*Silybum Marianum L.*). *Acta Alimentaria*, 44 (2): 304–310.

[68] Zhang, Z.S., Wang, S., Liu, H., Li, B.Z. & Che, L. (2020). Constituents and thermal properties of milk thistle seed oils extracted with three methods. *LWT*, 126: 109282.

[69] Mahran, M. Z. & Elhassaneen, Y. A. (2023). A Study of the Physical, Chemical, Phytochemical and Nutritional Properties of Wild Silybum marianum L. Seeds Oil to Investigate Its Potential Use to Boost Edible Oil Self-Sufficiency in Egypt. *Alexandria Science Exchange Journal*, 44, (1): 81-91. [DOI: 10.21608/asejaiqjsae.2023.292950].

[70] Malekzadeh, M., Mirmazloum, S.I., Anguorani, H.R., Mortazavi, S.N. & Panahi, M. (2011): The physicochemical properties and oil constituents of milk thistle (*Silybum marianum* Gaertn. cv. Budakalászi) under drought stress. *J. Med. Plants Res, 5*: 1485-1488.

[71] Dabbour, I.R., Al-Ismail, K.M., Takruri, H.R. & Azzeh, F. S. (2014). Chemical characteristics and antioxidant content properties of cold pressed seed oil of wild milk thistle plant grown in Jordan. *Pakistan Journal of Nutrition*, 13: 67-78

[72] Tungmunnithum, D., Thongboonyou, A., Pholboon, A. & Yangsabai, A. (2018). Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines*, 5(3): 93.

[73] Gharib, M., Hanan, A. Radwan. & Elhassaneen, Y. (2022). Nutrients and Nutraceuticals Content and *In Vitro* Biological Activities of Reishi Mushroom (*Ganoderma lucidum*) Fruiting Bodies. *Alexandria Science Exchange Journal*, 43(2): 301-316.

[74] Gillessen, A. & Schmidt, H. H.-J. (2020). Silymarin as Supportive Treatment in Liver Diseases: A Narrative Review. *Advances in Therapy*.

[75] Feher, J. & Lengyel, G. (2012). Silymarin in the prevention and treatment of liver diseases and primary liver cancer. *Current Pharmaceutical Biotechnology*, 13(1): 210–217.

[76] Clichici, S., Olteanu, D., Nagy, A. L., Oros, A., Filip, A. & Mircea, P. A. (2015). Silymarin inhibits the progression of fibrosis in the early stages of liver injury in CCl(4)-treated rats. *Journal of Medicinal Food*, 18(3): 290–298.

[77] Ni, X. & Wang, H. (2016). Silymarin attenuated hepatic steatosis through regulation of lipid metabolism and oxidative stress in a mouse model of nonalcoholic fatty liver disease (NAFLD). *American Journal of Translational Research*, *8*(2): 1073–1081.

[78] Badawy, N. (2021)."Antioxidant activities of Milk Thistle (Silybum marinum) and its effects on liver disorders induced by carbontetrachloride ". *MSc. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt.*

[79] Badawy, R. (2009). " Microbiolgical and Chemical Treatment Of Some Edible Oils Produced Be Deep-Fat Frying Process" M.Sc. *Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt.*

[80] Aly, A., Elbassyouny, G. & Elhassaneen, Y. (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, 2017, Sharm ElSheikh, Egypt*.

[81] Elhassaneen, Y., Goarge, S., Sayed, R. & Mahmoud, S. (2017). Comparative studies on three vegetable oils commonly consumed in Egyptian local markets. *Journal of Home Economics*, 27 (1): 139-150.

[82] Wroniak, M., Kwiatkowska, M. & Krygier, K. (2006). Characteristic of selected cold pressed oils. *Food Sci. Technol. Qual*. 2: 46–58.

[83] Elhassaneen, Y., Sabry, S. & Reham, B. (2016-a). Antioxidant activity of methanol extracts from various plant parts and their potential roles in protecting the liver disorders induced by benzo(a)pyrene. *Public Health International*, 2 (1): 38-50.

[84] Elhassaneen, Y., Ragab, S. & Mashal, R. (2016-c): Improvement of bioactive compounds content and antioxidant properties in crackers with the incorporation of prickly pear and potato peels powder. *International Journal of Nutrition and Food Sciences.* 5 (1): 53-61.

[85] El-Gamal, N., El-Dashlouty, M. & Elhassaneen, Y. (2020). Effects of brown algae (Sargassum subrepandum) consumption on obesity induced changes in oxidative stress and bone indices. *Journal of Home Economics*, 30 (4):687-708.

[86] Antolovich, M., Prenzler, P., Patsalides, E., Mcdonald, S. & Robards, K.J.T.A. (2002). Methods for testing antioxidant activity. *National Library of Medicine*, 127 (1): 183-198.

[87] Kahkonen, MP., Hopia, AI., Vuorela, HJ., Rauha,J., Pihlaja,K., Kujala,ST. & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* 47: 3954-3962.

[88] Fayez, S.A.(2021). "Effect of brown algae on obesity and its complications induced by high fat diets in rats*". PhD Thesis in Nutrition and Food Science, Faculty of Specific Education, Port Saied University, Port Saied, Egypt*

[89] Shinichi, T. (2011). Carotenoids in algae: distributions, biosyntheses and functions. *Mar. Drugs, 9*:1101-1118

[90] Aly, A., Ghada, M. Elbassyouny. & Elhassaneen, Y.(2018). 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" October, 2018, Sharm ElSheikh, Egypt*, 1-24, 24-27.

[91] Aviram, M., Dornfeld, L., Rosenblat, M., Volkova, N., Kaplan, M. & Coleman, R. (2000). Omegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. American Journal of Clinical Nutrition, 71: 1062–1076.

[92] Majid, S., Khanduja, K.L., Gandhi, R.K., Kapur, S. & Sharma, R.R. (1991). Influence of ellagic acid on antioxidant defense system and lipid peroxidation in mice. *Biochem. Pharmacol*, 42 (7): 1441-1445.

[93] Elbasouny, G., Shehata, N. & Elhassaneen, Y. (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* ", 24-26 February 2019, Faculty of Specific Education, Ain Sokhna University, El-Ain El-Soghna, Egypt

[94] Laranjinha, J., Almeida, L. & Madeira, V. (1994). Reactivity of dietary phenolic acids with peroxyl radicals: antioxidant activity upon low-ensity lipoprotein peroxidation. *Biochem. Pharmacol*.48 (3): 487-494.

[95] Sadeek, A.M., & Abdallah, E.M.(2019). Phytochemical Compounds as Antibacterial Agents A Mini Review. *Saudi Arabia Glob J. Pharmaceu Sci*. 53(4).

[96] Chisolm, G. & Steinberg, D. (2000). The oxidative modification hypothesis of atherogenesis: an overview. *Free Radical and Biological Medicine*, 28: 1815–1826.

[97] Li, Y., Changjiang, G., Jijun, Y., Jingyu, W., Jing, X. & Shuang, C.(2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry*, 96 : 254–260

[98] *Hong, W. & Cam, P. (2015). Atherosclerosis: Risks, Mechanisms, and Therapies*, Published by John Wiley & Sons, Inc., Hoboken, NJ

[99] Guo, Y., Wang, S., Wang, Y. & Zhu, T. (2016). Silymarin improved diet-induced liver damage and insulin resistance by decreasing inflammation in mice. *Pharmaceutical Biology*, 54:2995– 3000.

[100] Yao, J., Zhi, M. & Chen, M. (2011). Effect of silybin on high-fat-induced fatty liver in rats. *Brazilian J Med Biol Res*, 44: 652– 659.

[101] El-Safty, A.E. (2012). Production of some important nutritional and functional compounds from the by-products of food processing companies *" Ph.D. Thesis in Nutrition and Food Science, Faculty of Home Economics*.

[102] Bonet, M., Jose, A., Joan, R. & Andreu, P. (2015): Carotenoids and their conversion products in the control of adipocyte function, adiposity and obesity. *Archives of Biochemistry and Biophysics*, 572: 112–125.

[103] 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 Science), Faculty of Specific Education, Port Said University, Egypt.*

[104] Mahran, M.Z., Ghada M. Elbassyouny. & Yousif A. Elhassaneen. (2018-a). Preventive effects of onion skin powder against

hepatotoxicity in rats treated with benzo(*a*)pyrene. Proceeding of the Annual Conference (13th Arab; 10th International), 11-12 April, Faculty of Specific Education, Mansoura University, " *Higher Education in Egypt and the Arab World in the Light of Sustainable Development Strategies", Mansoura, Egypt.*

[105] Saad A. S. Hallabo., Shahinaz A. Helmy., Yousif Elhassaneen. & Mohamed Shaaban. (2018). Utilization of mango, onion and potato peels as sources of bioactive compounds in biscuits processing. *BIOSCIENCE RESEARCH, 1*5(4): 3647-3657.

[106] Pagana, K.D. & Pagana, T.J. (1997). Mosby's diagnostic and laboratory test references. *3 rd ed., Mosby-year Book, Inc., New York.*

[107] Sayed Ahmed, S., Shehata, N. & Elhassaneen, Y. (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.

[108] Elhassaneen, Y., Ragab, S., Abd El-Rahman, A. & Arafa, S. (2021-b). Vinca (Catharanthus roseus) Extracts Attenuate Alloxan-Induced Hyperglycemia and Oxidative Stress in Rats. *American Journal of Food Science and Technology*, 9 (4): 161-172 .

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

[110] Krecman, V., Skottova, N., Walterova, D., Ulrichová, J. & Simánek, V. (1998). Silymarin inhibits the development of diet-induced hypercholesterolemia in rats. *Planta Medica*, 64, 138-142.

[111] Di Sario, A., Bendia, E., Taffetani, S., Omenetti, A., Candelaresi, C., Marzioni, M. & Benedetti, A. (2005). Hepatoprotective and antifibrotic effect of a new silybin–phosphatidylcholine–vitamin E complex in rats. *Digest Liver Dis*, 37, 869-876.

[112] Haddad, Y., Vallerand, D., Brault, A. & Haddad, P.S. (2011). Antioxidant and hepatoprotective effects of silibinin in a rat model of nonalcoholic steatohepatitis. *Evid Based Complement Alternat Med*,164.

[113] Feng, B., Meng, R., Huang, B., Shen, S., Bi, Y. & Zhu, D. (2016). Silymarin alleviates hepatic oxidative stress and protects against metabolic disorders in high-fat diet-fed mice. *Free Rad Res*, 5 :314– 327

[114] Derosa, G., Romano, D., D'Angelo, A. & Maffioli, P. (2015). *Berberis aristata*/*Silybum marianum* fixed combination (Berberol® ) effects on lipid profile in dyslipidemic patients intolerant to statins at high dosages: A randomized, placebo-controlled, clinical trial. *Phytomedicine*, 22: 231-237.

[115] Heidarian, E. & Rafieian-Kopaei, M. (2012). Effect of silymarin on liver phoshpatidate phosphohydrolase in hyperlipidemic rats. *Bioscience Research*, 9: 59- 67.

[116] Sobolova, L., Skottova, N., Vecera, R. & Urbánek, K. (2006). Effect of silymarin and its polyphenolic fraction on cholesterol absorption in rats. *Pharmacological Research*, 53: 104-112.

[117] Radjabian, T. & Fallah Huseini, H. (2010). Anti-hyperlipidemic and anti-atherosclerotic activities of silymarins from cultivated and wild plants of *Silybum marianum* L. *With Different Content of Flavonolignans. Iran J Pharmacol Ther*, 9: 63-67.

[118] Gobalakrishnan, S., Asirvatham, S. S. & Janarthanam, V. (2016). Effect of Silybin on lipid profile in hypercholesterolaemic rats. *J Clin Diag Res*, 10: 01-05.

[119] Shaker, E., Mahmoud, H. & Mnaa, S.(2010) . Silymarin, the antioxidant component and Silybum marianum extracts prevent liver damage. *Food and Chemical Toxicology*, 48: 803-806.

[120] Shimaa, I., Ramadan, M.A. & Shalaby, N.A. (2011). El-Banna HA, Hepatoprotective and Antioxidant Effects of Silybum marianumPlant in Rats. *IJAVMS*, 5 (6): 541-547.

[121] Bencze-Nagy, J., Strifler, P., Horváth, B., Such, N., Farkas,V., Dublecz,K. & Pál, L.(2023) Effects of Dietary Milk Thistle (*Silybum marianum*) Supplementation in Ducks Fed Mycotoxin-Contaminated Diets. *Vet. Sci*., 10(2):100.

[122] Alsaggar, M., Bdour, S., Ababneh, Q., Tamam, E., Nidal, Q. & Karem, H.(2020). Silibinin attenuates adipose tissue inflammation and reverses obesity and its complications in diet-induced obesity model in mice. BMC Pharmacol Toxicol, 21 (1):8.