The Effectiveness of Dried Chard (*Beta vulgaris var. cicla*) Leaves Consumption on Obese Diabetic Rats

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Abstract

Overweight and obesity are one of the main lifestyle illnesses that lead to further health concerns and contribute to numerous chronic diseases. Diabetes mellitus is the collective term for heterogeneous metabolic disorders whose main finding is chronic hyperglycemia. The present study was carried out to investigate the effects of chard leaves on controlling body weight and hypoglycemia in obese-diabetic rats. Thirty-five male albino rats were used. Obesity was induced by feeding 28 rats a high-fat caloric diet (HFCD) for 4 weeks. Then, obese rats were injected with were subcutaneously injected by streptozotocin (STZ) at the dose of 60 mg/kg of b wt to induce-diabetes in rats. Healthy rats (7 rats) were injected with an equivalent amount of saline solution and kept as a negative control group. Obese-diabetic rats were divided into 4 groups. Group 2, the untreated obesity-diabetic group (ODRs), was kept as a positive control group. While the other 3 obesity-diabetic groups were treated by feeding on the supplemented HFCD with 5, 7.5, and 10% of chard leaves powdered in proportion to the diet. The quantitative estimate revealed that TPs and TFs content of chard leaves were 41.00±2.01mg GAE/g and 3.26±1.05 QE/g, respectively. The results discovered that untreated obese-diabetic rats had a significant decline in the mean values of FI, serum levels of HDL-c, and serum activity of SOD and GSH enzymes. While there is a significant rise in the mean values of FBW, BWG, BWG %, VFW, and AI, and levels of blood glucose, serum insulin, leptin, TC, TG, TL, LDL-c, VLDL-c, MDA, and activities of AST, ALT and ALP enzymes, compared to healthy control rats. Further, histopathological examination of pancreases of obese-diabetic rats from the positive control group showed hypertrophy and hyperplasia of β-cells of islets of langerhans associated with pyknosis of their nuclei. As well, liver sections have fatty changes of hepatocytes and congestion of hepatic sinusoids, vacuolization of hepatocytes, and necrosis of sporadic hepatocytes. Interestingly, obese-diabetic rats from treated groups fed on the supplemented diet with chard leaves at levels of 5, 7.5, and 10% had improvement in all the tested biological and biochemical parameters, as well as histological study of pancreas and liver. The efficiency of chard leaves in the treating of obese-diabetic rats was significantly augmented as the supplemental levels of chard leaves to HFCD were increasing. Finally, it was concluded that chard leaves have beneficial effects on obese-diabetic rats fed HFCD through the reduction of relative body weight gain, fat deposit tissue and serum lipids, liver function, and lipid peroxidation.

Keywords: Chard; Obesity; Diabetes Mellitus; HFCD, Streptozotocin Antioxidant enzymes.

INTRODUCTION

Obesity is defined as an unhealthy excess of body fat, which increases the risk of medical illness and premature mortality **(Saltzman, 2019)**. The World Health Organization defines obesity as an abnormal or excessive fat accumulation that may impair health, further clarifying that the fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended **(WHO, 2022)**.

The increasing prevalence of obesity, the adverse effects on human life are becoming more significant, and it has become a worldwide health problem. As well the prevalence of severe obesity will be increased by 130% (**Yao *et al*., 2024**). Raised obesity is a major risk factor for diseases as cardiovascular diseases, diabetes , musculoskeletal disorders (especially osteoarthritis) and cancers (endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon) (**WHO, 2024**).

Diabetes mellitus (DM) is the common term for heterogeneous metabolic disorders whose main finding is chronic hyperglycemia. The cause is either a disturbed insulin secretion or a disturbed insulin effect, or usually both (**Petersmann *et al*., 2019** and **Shaik *et al*., 2022**). It is associated with complications affecting the retina, nerves, heart and kidney functions (**Papatheodorou *et al*., 2018**).

Obesity and blood glucose levels are closely related, with obesity significantly increasing the risk of developing type 2 diabetes and other metabolic disorders. Excess body fat, particularly abdominal fat, can lead to insulin resistance, where cells become less responsive to insulin, causing blood glucose levels to rise. This can overwhelm the pancreas, leading to further complications and potentially type 2 diabetes (**Klein *et al*., 2022**).

Synthetic antidiabetic drugs can produce undesirable side effects such as a common side effect hypoglycemia, weight gain, and gastrointestinal adverse effects (**El-Sayed, 2011**). Natural plant materials which are being used as traditional medicine of functional foods are considered one of the good sources for a new drug or a lead to making a new drug. Nowadays, global interest in using plant medicines or functional foods and their main components in developing novel, effective medications with fewer adverse effects is rising and has potential advantageous applications for several disorders. Swiss chard is one of these nature plants which can be used as a traditional medicine of functional foods. Chard leaves are popular vegetables in the Mediterranean diet, which represent plentiful and inexpensive sources.

Chard plant (*Beta vulgaris L. var. cicla*) or (f*lavescens, Chenopodiaceae*). It has a thick, crunchy stalk that can be white or colorful and wide fanlike green leaves (**Rana, 2016**). Chard extract had been reported to have hypoglycemic activity due to its flavonoids content (**Mohammed *et al*., 2019** and **Mzoughi *et al*., 2019**). Chard has nutritional and functional therapeutic effects according to folk medicine due to its natural antioxidant and anticancer agents. (**Alsuhaibani and Alshawi, 2022**).

Aim of the Study

The present study was conducted to examine the effectiveness of chard (*Beta vulgaris var. Cicla*) leaves on the controlling of body weight and blood glucose levels in obese-diabetic rats.

MATERIALS AND METHODS

Materials:

Chard Leaves:

Fresh whole chard (*Beta vulgaris var. cicla*) plant (**Photo 1**) were purchased from local vegetable markets and were identified at the National Center for Agricultural Research, Cairo, Egypt.

Rats:

Thirty-Five adult male rats (Sprague Dawley Strain), weighing about 180±5g, were obtained from the Laboratory Animal Colony, Helwan, Egypt.

Basal Diet Components:

Casein, cellulose, choline chloride, D-L methionine, vitamins, minerals, and other components were purchased from Al-Gomhoriya Pharmaceutical Company, Cairo, Egypt. While, starch, soybean oil, and sucrose were obtained from the local market.

Chemicals and Kits:

Streptozotocin (STZ), kits for biochemical analysis and the other chemicals were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Methods:

Identification of Chard Plant:

Fresh whole chard plants were identified at the agriculture research center, Egypt.

Preparation of Chard Plant:

Fresh whole chard plants were cleaned from dust with flowing water to get free of any dirt or external bodies. As well, roots, stems and all invalid parts removed. Then, the leaves were dried in a drying oven vacuum at 50ºC. Afterward, a grinder mill and sieves were used to obtain a powder particle size of less than 0.4mm for all plant. The final powder was packaged in a closed bag and stored in the refrigerator at 5ºC until use.

Determination of Total Phenolic and Flavonoids Content:

The total phenol content of the plant leaves were determined using the Folin-Ciocaleau method (**Chun *et al*., 2003**). A grading curve of gallic acid was prepared and the results were determined from the regression equation of the calibration curve and expressed as mg gallic acid equivalents per kg of the plant (mgGAE/g). The total flavonoids content of the plant was determined by the aluminium chloride test (**Kim *et al.*, 2016**) using quercetin as standard and the results were calculated as mg quercetin equivalent/Kg of plant (mgQE/g).

Preparation of Basal Diet (AIN-93M) and High Fat Caloric Diet:

All components of the basal diet were mixed together to fulfil the desirable adequate dietary intake for keeping the health state of rats as confirmed by **Reeves *et al*., (1993).** It consisting of protein (14%), soybean oil (5%), mineral mixture (3.5%), vitamin mixture (1%), fiber (5%), sucrose (10%), choline chloride (0.25%) and the remainder have corn starch (up to 100%).

A high-fat caloric diet (HFCD) was prepared as described by (**Bhatt *et al*., 2006**). Briefly, the basal diet was supplied with 59% calories based on sheep fat and soybean oil, 21% calories from 25 carbohydrate and 20% calories from protein. After that, the high-fat diet was divided into four parts. The first part was a diet high in fat only, while the second, the third and the fourth parts were supplemented with the chard leaves powder at levels of 5, 7.5 and 10%,

respectively. Then diets are packaged in an enclosed bag and stored in the refrigerator at 5ºC for use.

Experimental Design and Grouping of Rats:

Thirty-five of rats were housed in the animal house at the Faculty of Home Economics, Helwan University in wire cages under adjustment environmental conditions of the temperature (22±4°C), relative humidity (45% to 50%) and light/dark cycle (12/12 hr). The food and water supplies were uninterrupted during the experimental period. Prior to the trial study, rats were kept for a week to acclimatize. The experiment was conducted in two stages, each lasting 4 weeks, as follows:

- **First Stage (induction of obesity):** In this stage, rats were divided into two main groups; the normal group (7 rats) fed on the basal diet, while the second main group (28 rats) fed on HFCD for four weeks to induce obesity.

- **Second Stage (induction of diabetes):** All of the obese rats (28) were subcutaneously injected by *streptozotocin* (STZ) at the dose of 60 mg/kg of the body weight as described by **Akbarzadeh *et al*., (2007).** Non-diabetic control rats were injected with an equivalent amount of slain solution. Subsequently, rats were kept for the next 24 hours on 10% glucose solution to prevent hypoglycemia. Seventy-two hours after injection with STZ, the diabetic rats are confirmed by measuring the 4-h fasting blood glucose level from the tail vein. Animals with a blood glucose level above 300 mg/dl were considered obese-diabetic an included in the experiment.

Then, healthy and obese-diabetic rats were divided into 5 groups (7 each) as follows:

Group 1: kept as a negative control group rats (- ve group) fed on basal diet alone.

Group 2: untreated obese-diabetic rats which kept as positive control group (- ve group) were fed on HFCD alone.

Group 3: Treating obese-diabetic rats with feeding on HFCD with added chard leaves in proportions of 5% of the diet.

Group 4: Treating obese-diabetic rats with feeding on HFCD with added chard leaves in proportions of 7.5% of the diet.

Group 5: Treating obese-diabetic rats with feeding on HFCD with added chard leaves in proportions of 10% of the diet.

Estimation of Feed Intake, Body Weight Gain and Percent Change in Body Weight Gain:

Feed intake (FI) was calculated every day during the second stage of the experimental period. The changes in body weight were determined by weighing the animals on a balance scale prior to the second stage of the experiment (IBW) and at the end of the experimental period (FBW). The biological value of the diet was assessed by the determination of its effect on body weight gain (BWG) and the percent change of body weight gain was calculated using the following formula:

BWG = FBW – IBW

% Change of body weight gain = BWG/IBW X 100

Determination of Visceral Fat Weight and Adiposity Index:

Visceral fat weight (g) and adiposity index were determined as described by **Taylor and Phillips, (1996)** using the following formulas:

Visceral Fat Weight (g) = epididymis fat + retroperitoneal fat + abdominal fat

Adiposity index % = total pad fat weights/final body weight ×100.

Blood Collection and Serum Separation:

At the end of the second stage of the experiment period, animals were fasted for 12-hr., except of water. Then rats were anaesthetized with diethyl ether and scarified. Blood samples were collected from the posterior vena cava into dry clean centrifuge tubes. Blood samples were left at room temperature to clot, and then centrifuged for 15 minutes at 4000 rpm for serum separation. Serum samples were carefully aspirated using a needle, transfers into dry, clean test tubes and frozen at -20°C for biochemical analysis. Visceral fat was separated and weighed to estimate the adiposity index. Pancreas and liver from all animals were removed immediately, washed with saline solution, dried, and immersed in 10% buffered formalin for histopathology examination.

Biochemical Assay:

Estimation Levels of Blood glucose, Insulin and Leptin Hormones:

At the end of experimental period and after the rats had been fasted for 12 hrs., blood samples were collected by tail vein of the rats and blood glucose levels were measured immediately by using a single touch Glucometer (Ascensia ENTRUST, Bayer).

Serum concentrations of insulin was estimated by using a specific antibody radioimmunoassay (RIA) kits according to the described methods by **Posario, (2010).** Serum level of leptin hormone was determined using Enzyme-linked immunosorbent assays (ELISA) as described by **Xiong *et al*., (2005).**

Estimation of Serum Levels of lipid profile:

Serum levels of total cholesterol (TC), triglycerides (TG), total lipid (TL), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c) were estimated using commercial reagent kits (Biomed diagnostic, Egypt) as described by **Zollner and kirsch (1962), Friadwald *et al*., (1972), Vassault *et al*., (1986), Hostmark *et al*., (1991) and Young, (2001),** respectively. While, respectively. Very low density lipoprotein cholesterol (VLDL-C) was calculated using Friedewald's formula.

VLDL-c (mg/ dL) = TG/5

Estimation of Liver Functions:

The serum activity of Aspartate aminotransferase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) enzymes were colorimeters quantified utilizing kits (Diamond Co, Hanover, Germany) in line with the instructions of **Young (2001)** for AST and ALT assay, **Young, (2001)** for ALP assay. The biometrics were quantified using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) adjusted at 505 for AST, ALT and ALP.

Estimation of Serum Oxidative Stress Marker:

Malondialdehyde (MDA) as an oxidative stress marker was assayed quantitatively in serum using the MDA assay kit by a spectrophotometric method (ABCAM, UK). The MDA in the sample reacts with thiobarbituric acid (TBA) to generate a MDA-TBA adduct. The MDA-TBA adduct is quantified colorimetrically (OD = 532 nm). This assay detects MDA levels as low as 1 nmol /well colorimetrically **Ohkawa *et al*., (1979).**

Estimation of Serum Activity of Antioxidant Enzymes:

The procedure that is used for the evaluation of catalase (CAT) activity depends on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The formaldehyde produced is measured spectrophotometrically at 540 nm as described by **Wheeler *et al*., (1990)**.

The standard technique to assay the activity of SOD is that the kits used use an enzyme linked immunosorbent assaydouble antibody principle. The color change is measured spectrophotometrically at 450 nm as described by **Wheeler *et al*., (1990)**. The serum activity of GSH was assayed according to the kit's instructions as described by (**Ceballos-Picot *et al*., 1992**) using spectrophotometrically at 340nm.

Histopathological Examination:

Pancreas and liver of all the scarified rats were cleaned, dried and immersed in 10% formalin solution. Then, sections of both pancreas and liver were trimmed, washed and dehydrated in ascending grades of alcohol. Specimens was being then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns' thickness, and stained with Heamtoxylin and Eosin stain for examination as described by **Carleton, (1979).**

Statistical Analysis:

Data was evaluated statistically using computerized SPSS package program (SPSS 22.00 software for Windows) by one-way analysis of variance (ANOVA). The obtained data was expressed as Mean ± SD and the significant difference among means was estimated at $p<0.05$**.**

RESULTS AND DISCUSSIONS

Total Phenols and Flavonoids Content:

The total phenols (TPs) and flavonoids (TFs) content of chard leaves. The quantitative estimate revealed that TPs and TFs content were41.00±2.01mg GAE/g and 3.26±1.05 QE/g, respectively. In the same context **Hamdiken and Kechrid, (2017)** showed that TPC and TFC of 80% methanolic extract of chard leaf were 31.23 mg GAE/g DE and 6.85 mg QE/g DE, respectively. On the other hand, TPC of 50% methanolic extract of chard leaf was 11.04 mg GAE/g DW as reported by (**Moyo *et al*., 2018**). As well as, **Zein *et al*., (2015)** stated that TPC and TFC of chard leaf varied according to the type of solvent used in extraction, which were 1.26 mg GAE/g FW, 0.29 mg QE/g FW and 1.92 mg GAE/g FW, 0.19 mg QE/g FW in aqueous and methanolic extracts, respectively. The variation in phenolic and flavonoid content may be related to several factors that influence the total phenolic such as environmental factors, harvest period, variety, chemical composition, maturity stage, growing condition, soil state and type of solvents used for extraction, as reported by **Siwak *et al*., (2013**). Additionally, several factors affect the bio-accessibility of polyphenols including the chemical state of the compound, its release from the food matrix,

possible interactions with other food components and the presence of suppressors or cofactors (**Parada and Aguilera, 2007**).

Table (1):Total phenols and total flavonoids of chard leaves.

sample (100g) / Parameters	Chard leaves.
Total phenols	41.00±2.01mg GAE/g
Total flavonoids	3.26±1.05 QE/g

GAE/g: Gallic Acid Equivalent per gram, **QE/g** :Quercetin Equivalents per gram.

The Effect of Supplemented Diet with Chard Leaves on FI and BW in Obese-Diabetic Rats:

The effect of supplemented diet with chard leaves on feed intake (FI), final body weight (FBW), body weight gain (BWG) and body weight gain % (BWG%) in obese-diabetic rats results are recorded in **Table (2)** . The obtained results showed that the positive control group had a significant decrease ($p<0.05$) in the mean value of FI and significant ($p<0.05$) increase in the mean values of in FBW, BWG and BWG %, compared to the negative control group. The current results was compatible with **Rezq and Elgazar, (2017)** who recorded that rats fed on HFCD had a significant increase in body weight gain, compared to normal rats fed on the normal basal diet. Additionally, **Ogungbemi *et al*., (2017)** reported that a high-fat diet caused a significant increase in FBW and Feed efficiency ratio (FER) with a significant decrease in FI, compared to the rats fed on normal diets. Also, **Abdel-Rahman *et al*., (2020) and Mabrouk, *et al.,* (2020)** showed significant higher FBW of rats fed on an HFD compared to that of fed rats on a normal basal diet. Also **Ibrahim *et al*., (2022)** revealed feeding rats on HFCD caused significant increased ($P<0.05$) in FBW, BWG and RWG and decreased ($P<0.05$) in FI. This noticing provides that the increase in body weight is independent of the amount of food consumed by the rats, but its caloric content.

Interestingly, obese-diabetic groups (treated groups) fed on the supplemented diet with chard leaves at levels of 5, 7.5, and 10% had no significant changes ($p<0.05$) in the mean values of FI. While there had been a significant decrease ($p<0.05$) in the mean values of FBW, BWG and BWG %, compared to the untreated obese-diabetic rats (+ ve control group) fed on the HFCD alone.

So, the obtained results showed that the effectiveness of chard leaves on the improvement of FI and BWG in obese-diabetic rats was increased with increasing the added levels of chard leaves.

This results are compatible with **Bakry, (2006)** who found that feeding rats with diets containing chard leaves caused a relative reduction in body weight

gains and adipose tissue compared with those fed control diet. According to the results of **Gamba *et al*., (2021)** who reported that the chard leaves are rich in all kinds of flavonoids. Dietary flavonoids can reduce fat and carbohydrate intake by regulating their hydrolysis and absorption in the gastrointestinal tract **(Oteiza *et al*., 2018).** Additionally, **Franzoni *et al*., (2024)** revealed that chard higher content of fiber. Dietary fiber affects all parts of digestive tract. Dietary fiber in foods requires chewing so eating high fiber foods may slow down the entire digestive process. Results with isolated fibers vary with not all fibers slowing gastric emptying. Once fiber gets to the stomach it slows gastric emptying and enhances satiety (**Willis *et al*., 2011)**. Also, epidemiologic studies support that dietary fiber (plant leaves) intake strongly prevents obesity and is inversely associated with body fat and body mass index at all levels of fat intake **(Cruz-Bravo *et al*., 2011).** High-fiber foods have much less energy density compared with high-fat diet and can displace energy. Eating equal weight of high-fiber food increases satiety. The bulking and viscosity properties of dietary fiber are mainly responsible for the influencing satiety **(Slavin, 2005).**

Table (2): The effect of supplemented-HFCD with chard leaves on FI, FBW, BWG and BWG% in obese diabetic-rats.

Parameters / Groups		FI (g)	BW after induction of obesity (g)	FBW (g)	BWG (g)	BWG (%)
Normal control group		13.00±0.82^{a}	220.29±1.25^{c}	250.30±2.75^{d}	30.01±3.65^{d}	13.62±1.13^{d}
Positive control group		11.00±0.82^{b}	334.86±3.29^{a}	403.57±2.94^{a}	68.71±2.82^{a}	20.52±1.63^{a}
Treated obese-diabetic groups with chard at levels of:	5.00%	11.28±0.75^{b}	332.86±1.77^{ab}	365.57±2.07^{b}	32.86±2.73^{b}	9.87±0.86^{b}
	7.50%	11.42±0.79^{b}	332.71±2.14^{ab}	344.00±2.65^{c}	11.29±2.42^{c}	3.39±1.28^{c}
	10.0%	11.57±0.53^{b}	332.57±3.05^{ab}	342.71±2.14^{c}	8.71±2.11^{c}	2.62±1.25^{c}

Results are expressed as mean ± SD; Means with different superscript letters in the same column are significantly different at ($P<0.05$). **FI:** Feed Intake; **IBW:** Initial body weight; **FBW:** Final Body Weight, **BWG:** Body Weight Gain; **HFCD:** High Fat and Calories Diet

The Effect of Supplemented Diet with Chard Leaves on VFW and AI in Obese-Diabetic Rats:

Tableted results in **Table (3)** illustrated the effect of HFCD alone and supplemented HFCD with chard leaves on visceral fat weight (VFW) and adiposity index (AI) in obese-diabetic rats. The recorded results showed that the positive control group fed on HFCD had a significant increase ($P<0.05$) in the mean value of VFW and AI levels, compared to the negative control group fed on basal diet. The obtained results agreed with **Ibrahim *et al*., (2022)** who

showed that rats fed on HFCD had a significant (P<0.05) increase in VFW (g) and AI, compared to that of the fed rats on a normal basal diet. As well, **Rezq *et al*., (2024)** showed that NASH rats fed on HFCD had a significant (P<0.05) increase in VFW g and AI, compared to rats fed on the basal diet. In addition, obesity as showed in the present results is characterized by increased adipose tissue mass that results from both increased fat cell number and increased fat cell size (**Lafontan and Langin, 2009**). Abiogenesis is a part of the adipocyte differentiation process from pre-adipocyte precursors into mature adipocytes with the formation and enlargement of intracellular lipid droplets (**Ali *et al*., 2013**). This process is associated with the development of obesity. Excess energy intake and reduced energy expenditure results in abnormal excessive growth of white adipose tissue, which can lead to the development of obesity in rats (**Jo *et al*., 2009**). These results were confirmed by the significant (P<0.05) increase in visceral fat weight (VFW), relative weight of visceral fat % and adiposity Index (AI), compared to that of the fed rats on a normal basal diet (normal rats) in the current study.

On the other hand, feeding obese-diabetic rats the HFCD with added the three different levels of chard leaves caused a significant decrease (P<0.05) in the mean value of VFW and AI levels, compared to the positive control group fed on HFCD only. The lowest of VFW and AI values were more detected in obese-diabetic rats with increasing added levels of chard leaves.

Bakry, (2006) showed that adding dry Swiss chard leaves can reduce the harmful effects of high lipid diet on weight gain and deposit fat in rats model. As well indirect evidence from both epidemiologic and short-term experimental studies suggested a beneficial role of a high fiber diet in weight control (**Liu, 2002**). Dietary patterns play an important role in the control of body weight. Such specific eating patterns could help in reducing the rate of weight gain (**Drapeau *et al*., 2004**).

Additionally, study on rats fed a high-fat diet and supplemented with chard leaves (*Beta vulgaris L. var. cicla*) have shown potential benefits in reducing body fat and improving related metabolic markers. Specifically, chard extract has been linked to decreased fat accumulation. These findings suggest that chard could be a valuable dietary component for mitigating the adverse effects of obesity and high-fat diets (**Zeineb *et al*., 2018**). Flavonoids might downregulate the synergistic interaction between insulin and leptin signaling in the inflammatory processes (**Araújo *et al*., 2015**). Particularly, considering the anorectic activity of leptin, propolis has potential to attenuate feeding and subsequently prevent obesity (**Washio *et al*., 2015**).

Table (3): The effect of supplemented-HFCD with chard leaves on VFW and AI in obese diabetic-rats

Parameters / Groups		VFW (g)	AI (%)
Normal control group		9.29±0.49^{c}	2.54±0.13^{c}
Positive control group		14.01±0.82^{a}	3.48±0.21^{a}
Treated obese-diabetic groups with chard at levels of:	5.0%	12.02±0.82^{b}	3.29±0.18^{b}
	7.5%	11.45±0.53^{b}	3.33±0.16^{b}
	10.0%	10.01±0.82^{c}	2.92±0.22^{bc}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **VFW:** Visceral fat Weight**; AI:** Adiposity Index; **HFCD:** High Fat and Calories Diet**.**

The Effect of Supplemented Diet with Chard Leaves on the Levels of Blood Glucose and Serum Insulin and Leptin hormones in Obese-Diabetic Rats:

The effect of HFCD and the chard leaves at levels 5, 7.5, and 10% on blood glucose levels as well as serum insulin and leptin hormone levels in obese-diabetic rats is recorded in **Table 4**. The presented results revealed that the positive control group had a significant increase (P<0.05) in the mean values of blood glucose levels and serum insulin and leptin hormone levels compared to the negative control group. This result agreed with **Kusunoki *et al*., (2000)** who showed hyperglycaemia, dyslipidaemia and hyperinsulinaemia in rodents fed a high-fat diet. **Srinivasan *et al*., (2004)** revealed that the feeding on high-fat diet for a period of 30 days increased levels of serum insulin and insulin resistance. Some previous studies revealed that hyperinsulinemia and insulin resistance are common features of obesity in experimental animals **(Amin and Nagy, 2009).** As well **Unger and Scherer, (2010)** demonstrated that insulin resistance in humans can be linked to lifestyle and can be notice more as a cause of lipid deposition in a caloric excess**.**

Saravanan *et al*., (2014) showed that rats fed on a high-fat diet had high serum leptin hormone levels when compared with those fed on a normal basal diet. Disruption of leptin/leptin receptor signaling results in morbid obesity and severe metabolic disease (**Zhang and Chua, 2011**). **Handjieva-Darlenska and Boyadjieva, (2009)** revealed that the diet consisting of more fat might have accounted for the elevated levels of leptin. Leptin is a common protein produced by the adipose tissue and highly correlates with body fat, suggesting that obese persons are insensitive to endogenous leptin production. It is a key fat-derived regulator of food intake and energy expenditure and its secretion

levels were usually positively correlated with the extent of the triglyceride stores in adipocytes (**Staiger and Haring, 2005**).

In comparison to the positive control group, obese-diabetic groups fed the supplemented HFCD with the three different levels of chard leaves (5, 7.5 and 10%) have significant ($p<0.05$) decrease in blood glucose levels and serum insulin and leptin hormone levels. The lowest in blood glucose levels and serum insulin and leptin hormone levels were better noticeable with increasing the added levels of chard leaves as shown in **Table 4.** These findings at the same line with **Bolkent *et al*., (2000)** showed that administration of chard aqueous extract caused an increase in the number of ß- cells of Langerhans cells and in the secretory granules, together with many hypertrophic Golgi apparatus and granules of low densities. The extract reduced the blood glucose value in streptozotocin-induced hyperglycemic animals and significantly increased body weight in comparison to the diabetic group. They reported that chards may decrease blood sugar by increasing insulin secretion from B-cells of the pancreas. As a result, it can be assumed that plant therapy can provide blood glucose homeostasis and can cause regeneration of B-cells of endocrine pancreas. Additionally, **Gezginci-Oktayoglu *et al*., (2014)** reported that chard extract at 2 g/kg bw/daily caused a significant reduction in blood glucose and increased glycogen levels in the liver of the rats. **Kabir *et al*., (2015)** showed that aqueous fraction of beta vulgaris ameliorates hyperglycemia in diabetic mice due to enhanced glucose stimulated insulin secretion, mediated by acetylcholine and Glucagon like Peptide-1 and elevated glucose uptake via increased membrane bound GLUT4 transporters. Also, **Hamdiken and Kechrid, (2017)** revealed that oral administration of Beta vulgaris extract at 500 mg/kg bw to diabetic rats significantly decreased serum glucose. Chard has antioxidant activity due to its high phenolics, flavonoids, and proline, that protective effects of chard against pancreatic complications (**Trifunovic *et al*., 2015**). Several dietary flavonoids improve insulin sensitivity, inhibit obesity-related oxidative stress, improve the redox balance in affected individuals and thus improve macronutrient metabolism (**Gentile *et al*., 2018**). Research and clinical studies have provided evidence for the health benefits of flavonoids in treating and preventing diabetes due to their strong antioxidant and anti-inflammatory properties (**Oliveira *et al*., 2022**).

Dietary fiber intake are linked to less type 2 diabetes risk (**Reynolds *et al*., 2020**). It is generally accepted that an important mechanism is that fiber will slow glucose absorption and improve other risk factors with adults with prediabetes or diabetes. The role of the gut microbiota and gut microbial metabolites may be an important mechanism for fiber's ability to prevent obesity (**Canfora *et al*., 2019**). The chard is main edible sources of betalains in nature (**Kugler, *et al*., 2004**). **Dhananjayan *et al.,* (2017**) reported that oral

treatment with betalains in diabetic rats was able to restore blood glucose and insulin levels to normal ranges. This effect is a consequence of the activation induced by betalains on liver glycolytic enzymes. In addition, there was a significant reduction in the activity of gluconeogenic enzymes. Similary, conducted study by **Indumathi *et al*., (2018)** reaffirmed the antihyperglycemic capabilities of betalains. Treatment with betalains managed to restore normal blood glucose and insulin values.

Table (4): The Effect of Supplemented Diet with Chard Leaves on the Levels of Blood Glucose and Serum Insulin and Leptin hormones in Obese-Diabetic Rats

Parameters / Groups		Blood Glucose (mg/dl)	Insulin (mg/dl)	Leptin (ng/ml)
Normal control group		64.43±1.62^{e}	12.50±0.12^{e}	3.95±0.08^{e}
Positive control group		127.00±3.37^{a}	21.3±0.81^{a}	11.51±0.21^{a}
Treated obese-diabetic groups with chard at levels of:	5%	110.86±1.35^{b}	18.61±0.13^{b}	8.32±0.12^{b}
	7.5%	94.14±2.79^{c}	16.44±0.15^{c}	6.01±0.38^{c}
	10%	71.43±1.99^{d}	14.56±0.24^{d}	4.23±0.12^{d}

Results are expressed as mean ± SD; Means with different superscript letters in the column are significantly different at ($P<0.05$).

The Effect of Supplemented Diet with Chard Leaves on Lipid Profile in Obese-Diabetic Rats:

Results in **Table 5** exhibit the effect of feeding obese-diabetic group on the HFCD alone and HFCD- supplemented with chard leaves on the serum levels of total cholesterol (TC), triglycerides (TG), total lipids (TL), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c) and very low density lipoprotein cholesterol (VLDL-c). In comparison to the negative control group fed on the basal diet, positive control group (obese-diabetic rats) fed on the HFCD alone have a significant ($P < 0.05$) increase in serum concentrations of TC, TG, TL, LDL-c and VLDL-c, and decreased serum HDL-c.

Dyslipidemia is another important lineament in the manner of development of obesity which characterized by hyperlipidemia, hypertriglyceridemia with increased level of LDL-c and VLDL-c. Hypercholesterolemia is one of the risk factors for the emergence of atherosclerosis, which is an inflammatory disorder in artery walls characterized by the formation of atheroma **(Newby *et al.,* 2014).** This outcome were in alignment with **Kusunoki *et al*., (2000)** who proved the incidence of dyslipidemia in rodents fed on a high-fat diet. Also, these consequence confirmed by the results of **Sumiyoshi *et al*., (2006)** who reported

that HFD admission bring to the induction of hyperlipidemia, hypertension, glucose intolerance and atherosclerosis. As well as, the obtained results was compatible with **Ani *et al*., (2020)** who revealed that diabetes caused an increase in the lipid profile of the rats. Also, the present results were in accordance with **Rezq and El-Khamisy, (2011)** who showed that high-fat diet results in dyslipidaemic changes by increase serum TG, VLDL, TC and LDL-c and decrease serum HDL-c levels**.** Additionally**, Rezq *et al*., (2024)** showed that the parameters of serum TL, TC, TG, LDL-c, and VLDL-c levels were significantly increased in NASH rats fed HFCD, while HDL-c levels were decreased, compared to that of the normal rats fed on a normal basal diet.

In comparison to the positive control group, combination of HFCD with the three different levels of chard leaves resulted in significantly ($P < 0.05$) lower serum levels of TC, TG, TL, LDL-c and VLDL-c, and a raise in serum HDL-c. The preferable improvement in serum levels of lipid profiles was discovered with feeding obese-diabetic groups HFCD-supplemented with the higher levels of chard leaves as shown in **Table 5.** These findings were in the same line with **Bakry, (2006),** which showed that adding dry form of chard leaves can reduce the harmful effects of a high lipid diet on weight gain and deposit fat, as well as total lipids, total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol, which may be due to the effect of different components. **Sener *et al*., (2002)** reported that chard leaves, also known as Swiss chard, have shown potential in improving lipid profiles, particularly in reducing levels of harmful cholesterol and triglycerides, while potentially increasing beneficial HDL cholesterol. This effect is attributed to the antioxidant and anti-inflammatory properties of chard, which can help mitigate the negative impacts of high-fat diets and related metabolic issues. **Shaaban *et al*., (2021)** reported that supplementation of Gamma-irradiated Chard Leaves along with HFD resulted in remarkable protection against HFD-complications and that evidenced by reduction in total cholesterol (TC), triglycerides (TG),Low-density lipoprotein-cholesterol (LDL-C) and very Low-density lipoprotein-cholesterol(VLDL-C),remarkable increase in high-density lipoprotein cholesterol (HDL-C). **Ustundag *et al*., (2016)** revealed that chard leaves is rich in minerals, vitamins and phytochemicals and is one of the healthiest vegetables. Main identified secondary metabolites are flavonoids, flavonoid glycosides, betalains and saponins. Flavonoids have an important role in improving metabolic processes as reflected by higher body weight gain **(Astrini, 2017).** From the last few decades, **Johnson *et al*., (2020)** suggested that dietary polyphenols have exhibited the potential of reducing plasma LDL-C levels. Also, **Sun *et al*., (2021)** found that the flavonoids, can reduce the levels of total cholesterol (TC), triglycerides (TG), and LDL-c, and increase the level of HDL-c in the blood. Flavonoids inhibit cholesterol uptake through multiple mechanisms and inhibit

intestinal cholesterol absorption mediated by NPC1L1.This is a newly found polyphenol function **(Nekohashi *et al*., 2014).** Flavonoids have received considerable attention for their lipolytic activity in vitro and mammals **(Alkhalidy *et al*., 2018).** Additionally, **Yahaghi *et al*., (2020)** attempted to find out the molecular mechanisms underlying betalains action on mice with hepatosteatosis. The disease was induced through a hyperlipid diet, producing excessive fat, increased blood cholesterol and triglyceride levels. All these changes were effectively attenuated after the intake of betalains.

Table (5): The Effect of Supplemented Diet with chard leaves on Serum TL, TC, TG, (HDL-c), (LDL-c) and (VLDL-c) Levels in Obese Diabetic Rats

Parameters / Groups		TC (mg/dl)	TG (mg/dl)	TL (mg/dl)	LDL-c (mg/dl)	HDL-c (mg/dl)	VDL-c (mg/dl)
Normal control group		116.57±2.07^{e}	84.57±1.35^{e}	574.57±2.37^{e}	54.37±2.04^{e}	54.29±1.80^{a}	17.03±0.44^{e}
Positive control group		273.43±3.99^{a}	186.14±1.35^{a}	742.2±0.38^{a}	202.63±3.86^{a}	33.57±0.98^{e}	37.23±0.27^{a}
Treated obese-diabetic groups with chard at levels of:	**5%**	193.14±1.77^{b}	155.29±2.29^{b}	684.86±2.27^{b}	124.80±1.73^{b}	37.43±0.98^{d}	31.06±0.46^{b}
	7.5%	162.57±1.62^{c}	103.57±1.72^{c}	655.00±2.77^{c}	103±1.49^{c}	38.86±0.90^{c}	20.71±0.34^{c}
	10%	135.00±20^{d}	96.29±2.50^{d}	614.00±2.08^{d}	75.60±1.45^{d}	40.14±0.90^{b}	19.26±0.50^{d}

Results are expressed as mean ± SD; Means with different superscript letters in the column are significantly different at (P<0.05). HFCD: High Fat and Calories Diet; TC: Total cholesterol; **TG**: Triglyceride; **TL:** Total Lipid; **HDL-c**: High Density Lipoproteins Cholesterol; **LDL-c**: Low Density Lipoproteins Cholesterol and **VLDL-c**: Very Low Density Lipoproteins Cholesterol.

The Effect of Supplemented Diet with Chard Leaves on activity of liver function enzymes AST, ALT and ALP in Obese-Diabetic Rats:

Since, hepatic enzymes AST, ALT and ALP are the most specific intracellular enzymes that are associated with cell leakage and serve as a marker of hepatocellular injury with greater grades of hepatic steatosis and fibrosis. The elevation in the hepatic enzymes may be attributed to an increase in the production of free radicals that initiate lipid peroxidation of membrane leading to loss of integrity of cell membranes and damage of hepatic cells. The metabolic processes resulting from a high-fat diet (HFD) can cause oxidative stress in mitochondria and the endoplasmic reticulum, as well as induce de novo lipogenesis and inflammation in liver cells (**Yang *et al*., 2019**).

Table (6) show the effect of feeding obese-diabetic groups of rats on chard leaves adding to HFCD at levels of 5, 7.5 and 10% on the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline

phosphates (ALP) enzymes. The present study revealed that HFCD alone caused a significant ($P<0.05$) increase in the serum activities of AST, ALT, and ALP enzymes in obese-diabetic rats, compared to that caused by normal basal diet in normal rats.

The current results agree with the results of **Huang *et al*., (2022)** who reported that the high-fat diet significantly elevated the serum activities of levels of AST and ALT enzymes in male rats. As well, the results of **Sunmonu and Afolayan (2013)** revealed that diabetes was also associated with a significant increase in serum ALT and AST activities. AST and ALT are well-known hepatic enzymes that cause liver cell damage in case of elevated serum activity, as observed in diabetic rats. Also, the results of **Ibrahim *et al*., (2022)** showed that HFCD in obese rats caused a significant ($P<0.05$) increase in serum activity of AST, ALT, ALP enzymes, compared to rats fed on the basal diet. **Meli *et al*., (2013)** observed that Feeding rats HFD for long time led to increase expression of inflammatory factors mRNA such as IL-6 and TNF- due to increase liver tissue damage. **Amin and Nagy, (2009)** who postulated that presence of extra FFA's in portal blood could cause inflammation within hepatic cells, and furthermore release pro-inflammatory cytokines, leading to more hepatocyte injury, and affecting the liver cell's permeability with over releasing of hepatic enzymes to the blood stream. Also, **Tan *et al*., (2023)** reported that obese-diabetic rats exhibit significant liver dysfunction. These changes are closely linked to insulin resistance and metabolic syndrome.

In contrast, the added of chard leaves at the three different levels (5, 7.5, and 10%) to HFCD caused a significant decrease ($P<0.05$) in the serum activities of AST, ALT, and ALP enzymes in obese-diabetic rats as compared to those induced by HFCD alone. It was noticed that with increasing levels of the chard leaves added to HFCD, serum activities of AST, ALT, and ALP were lowered significantly. The obtained findings were in the same line with the results of **Hashem *et al*., (2016)** who found that the liver appearance and pathology of obese rats worsened; the levels of ALT and AST in serum were increased significantly. While in treated groups with chard leaves extract, the appearance and pathology of the liver were improved, and the levels of ALT and AST in serum were decreased significantly. **Ozsoy-Sacan *et al*., (2004)** have shown that chard extract can reduce levels of liver enzymes (ALT, AST, and ALP) and total lipid levels in diabetic rats, suggesting a protective effect on liver function. Also, **Jain and Singhai (2012)** mentioned that Chard could maintain the functional integrity of hepatocyte membrane, thus protecting the hepatocytes against ethanol toxicity and was also found to be effective in decreasing the leakage of ALT triggered by ethanol. Also, **Ertik *et al*., (2021)** reported that

Chard leaves extract has shown potential benefits for liver function in obese, diabetic rats. This is more likely attributed to the existence and joint activity of the phytocomponents of flavonoid and nonflavonoid origins, which have antioxidant properties (**Yahyaa *et al.,* 2015**).

Table (6): The Effect of Supplemented Diet with Chard Leaves on liver function enzymes AST, ALT and ALP in Obese-Diabetic Rats

Parameters / Groups		AST(U/L)	ALT(U/L)	ALP(U/L)
Normal control group		22.86±1.57^{e}	18.71±1.11^{d}	582.86±2.19^{d}
Positive control group		65.86±2.12^{a}	47.29±2.29^{a}	872.57±2.82^{a}
Treated obese-diabetic groups with chard at levels of:	5%	44.43±1.72^{b}	33.29±1.80^{b}	615.57±2.86^{b}
	7.5%	35.00±2.08^{c}	25.43±1.40^{c}	600.43±1.27^{c}
	10%	30.71±1.11^{d}	19.86±1.35^{d}	552.29±2.87^{e}

Results are expressed as mean ± SD; Means with different superscript letters in the column are significantly different at ($P<0.05$). **AST**: Aspartate transaminase; **ALT**: Alanine transaminase; **ALP**: Alkaline phosphate; **HFCD**: High Fat and Calories Diet.

The Effect of Supplemented Diet with Chard Leaves on serum levels of MDA, and activity of GSH and SOD enzymes in Obese-Diabetic Rats:

Table 7 represents lipid peroxidation as indicated by serum level of malondialdehyde (MDA) and activity of reduced glutathione (GSH) and superoxide dismutase (SOD) enzymes in normal rats, non-treated obese-diabetic group, and obese-diabetic groups which are treated by adding three different levels of chard leaves (5, 7.5, and 10%) to HFCD.

The present study provides a perfect correlation between serum lipid peroxidation products as indicator by MDA and the activity of antioxidant enzymes, which play an important role in the antioxidant system. Results showed that non-treated obese-diabetic rats (+ve group) have a significant increase at $p<0.05$ in the serum level of MDA and a decrease in the activity of SOD and GSH enzymes compared to normal rats (- ve group).

High-fat diets result in the release of free fatty acids by the action of lipoprotein lipase, which increases serum triglycerides and causes lipotoxicity,

which results in insulin receptor dysfunction. The release of excessive free fatty acids provokes lipotoxicity, as lipids and their metabolites create oxidative stress. As well, the decrease in serum activity of antioxidant enzymes, as seen in the serum of obese rats, can lead to the excessive availability of superoxide and peroxyl radicals, which in turn generate hydroxyl radicals, resulting in the initiation and propagation of more lipid peroxidation products **(Zhang *et al*., 2007)**. The existing result was agreed with the results of **Amirkhizi *et al*., (2007)** study that revealed that increasing the production of reactive oxygen species as well as reduced antioxidant defense mechanisms has been suggested to play a role in both humans and animal models of obesity. Furthermore, hypertriglyceridemia results in obese rats participating in the alteration of oxidant-antioxidant balance, suggesting an increase in the bioavailability of free fatty acids and lipid peroxidation. Also, hyperlipidemia induces oxidative stress and increases lipid peroxidation (**Moussa, 2008**). Therefore, lipid alterations have been considered as contributory factors to oxidative stress in obesity (**Leopold and Loscalzo, 2008**). **Denisenko and Novgorodtseva (2013)** showed that feeding animals on a high-fat diet inhibits the activity of blood antioxidant enzymes and elevates lipid peroxidation (MDA). In addition, the prooxidative effects have been documented as it decreases the activity of antioxidant enzymes such as SOD, CAT, and GPX and increases the concentration of malondialdehyde (MDA), which is the main marker of lipids peroxidation (**Capatina *et al*., 2020**). As well, elevated blood glucose levels can overcome the body's antioxidant defenses, such as SOD and CAT, leading to an accumulation of ROS (**Alipour *et al.,* 2012**). It is well-known that hyperglycemia causes metabolic disorders since it triggers “aberrant” pathways that promote oxidative stress in human tissues (**Lima *et al*., 2022**). Additionally, high blood glucose in rats leads to increased oxidative stress. This occurs might be due to high blood glucose levels triggering the production of reactive oxygen species (ROS) through various pathways, including glucose auto-oxidation, the polyol pathway, and the formation of advanced glycation end products. These ROS can damage cellular components, contributing to the development of complications associated with diabetes (**González *et al*., 2023**).

Whilst feeding obese-diabetic groups of rats on complement HFCD with the three different levels of the chard leaves has a significant decrease at $p<0.05$ in the serum levels of MDA , and an increase in the activity of antioxidant enzymes (SOD and GSH), compared with the non-treated obese-diabetic group fed on HFCD only.

The improvement in the serum concentration of MDA and activity of antioxidant enzymes was shown in the treated group with increasing the levels of chard leaves added to HFCD. These outcomes hint that chard could be a

valuable dietary component for managing oxidative stress and potentially alleviate some of the harmful effects associated with obesity and diabetes.

Various studies identified the existence of numerous bioactive components of the chard leaves as phytopigments, flavonoids, and minerals with antioxidant and immunomodulating properties (**Ivanović *et al.*, 2019**). Also, **Ustundag *et al*., (2016)** revealed that chard leaves are rich in minerals, vitamins, flavonoids, flavonoid glycosides, betalains and saponins. Therefore, chard (Beta vulgaris) is one of the functional foods that is considered a supplement of biologically active and phytochemical compounds that have phenolic acids, ascorbic acid, carotenoids, and flavonoids that have a high ability to scavenge free radicals (**Hajihossein *et al*., 2017**). Besides, it contains highly bioactive pigments known as betalains that are synthesized from the amino acid tyrosine into 2 structural groups: yellow–orange beta-xanthins and also the red–violet beta-xanthins (**Al Nouri *et al*., 2017**). Additionally, chard has been used in traditional medicine as a strong antioxidant, anti-diabetic, and hepato-protective agent because it contains various natural components, such as carotenoids, some fatty acids, phospholipids, glycolipids, polysaccharides, folic acid, vitamins C and E, and polyphenolic and thiol compounds (**Ustundag *et al*., 2016**).

Yarat *et al*., (2011) concluded that chard leaves, when consumed by obese-diabetic rats, have been shown to potentially decrease levels of MDA and increasing antioxidant activity. This suggests that chard could offer protective effects against the oxidative damage often associated with diabetes and obesity. These results agree with **Hamdiken and Kechrid (2017)**, who concluded that chard performed a positive role in lowering MDA levels and rising the GSH levels in exposed rats to ethanolic toxicity.

On the other hand, **Hajihossein *et al*., (2017)** revealed that the anti-oxidant and free radical-scavenging activities of chard could be attributed to its active components such as phenolic contents, flavonoids, glycosides, and saponins. Also, the amino acid proline, one of the chard components, is considered one component of the intracellular defense system against oxidative stress caused by free radicals and ROS (**Unsal *et al*., 2016**). As mentioned by **Gezginci-Oktayoglu *et al*., (2014)** admenistration of chard extract to hyperglycemic rats results in decreasing the oxidative stress (malondialdehyde formation) and increasing antioxidant defense (the activities of CAT, SOD and GSH). These findings suggest that chard extract might improve glucose response by increasing GLUT2 through Akt2 and antioxidant defense in the liver.

Table (7): The Effect of Supplemented Diet with Chard Leaves on serum levels of MDA, and activity of GSH and SOD enzymes in Obese-Diabetic Rats

Parameters / Groups		MDA (nmol/ml)	GSH (mmol/ml)	SOD (ng/ml)
Normal control group		67.43±2.51^{e}	4.53±0.20^{a}	952.57±1.51^{a}
Positive control group		192.71±1.83^{a}	2.25±0.02^{e}	414.86±2.54^{e}
Treated obese-diabetic groups with chard at levels of:	5%	141.57±2.82^{b}	2.98±0.05^{d}	696.86±1.57^{d}
	7.5%	101.43±0.98^{c}	3.93±0.05^{c}	816.29±3.95^{c}
	10%	83.86±3.02^{d}	4.41±0.01^{b}	894.29±2.5^{b}

Results are expressed as mean ± SD; Means with different superscript letters in the column are significantly different at (P<0.05). **HFCD**: High Fat and Calories Diet; **MDA**: malondialdehyde; **GSH**: reduced glutathione; **SOD**: superoxide dismutase.

Histopathological Examination:

1-Histopathological Examination of Pancreases:

Microscopic examination of pancreas sections from the normal group revealed no sign of histological structure variations as shown in **Photo (1)**. Nevertheless, pancreas sections of non-treated obese-diabetic rats from the positive control group showed hypertrophy and hyperplasia of β-cells of islets of langerhans associated with pyknosis of their nuclei (**Photo 2**). This result agreed with the results of **Laxmi *et al*., (2010),** who showed that there was extensive damage of the langerhans in alloxan-induced diabetic rats. This effect may be attributed to the higher blood glucose levels, which cause a deterioration of pancreatic β cells resulting from oxidative stress. Also, **Rezq, (2011)** revealed that alloxan-induced diabetic rats had hypertrophy and hyperplasia of β- cells of islets of langerhans associated with pyknosis of their nuclei. Recently, **Wickramasinghe *et al*., (2024)** reported that the major histopathological changes in the pancreas of HFD-fed STZ-induced diabetic rats were loss of pancreatic islets, pancreatic islet hypertrophy, and mild fatty change in the exocrine pancreas.

In contrast, pancreas sections of the treated obese-diabetic rats by feeding on 5% chard leaves added to HFCD had vaculations of acinar epithelial lining in the pancreas as shown in **Photo (3)**. As well, slight hypertrophy of islets of langerhans was shown in pancreas sections of rats from treated obese-diabetic groups by feeding on 7.5 and 10% chard leaves added to HFCD as shown in **Photo (4)**.

As mentioned by **Bolkent *et al.*, (2000)** plant therapy can provide blood glucose homeostasis and can cause regeneration of B-cells of endocrine pancreas. They also showed that administration of chard aqueous extract caused an increase in the number of ß- cells of Langerhans cells and in the secretory granules, together with many hypertrophic Golgi apparatus and granules of low densities. As well the extract reduced the blood glucose value in streptozotocin-induced hyperglycemic animals. They reported that chards may decrease blood sugar by increasing insulin secretion from B-cells of the pancreas.

Chard has antioxidant activity due to its high phenolic, flavonoids, and proline, that protective effects of chard against pancreatic complications (**Trifunovic *et al.*, 2015**). Therefore, antioxidants can have a beneficial effect on pancreatic β cells by neutralizing the oxidative stress. Normal β- cells compensate for insulin resistance by increasing glucose-stimulated insulin secretion or β -cell mass **Kaneto *et al.*, (2001)**. Additionally, **Helmy *et al.*, (2024**) Reported that chard leaf extracts exhibit possible effects in protecting pancreatic tissue and promoting regeneration of pancreatic beta cells in diabetic models. These effects are commonly linked to chard antioxidant, hypoglycemic properties, reduce lipid peroxidation, and enhance antioxidant levels in diabetic rats.

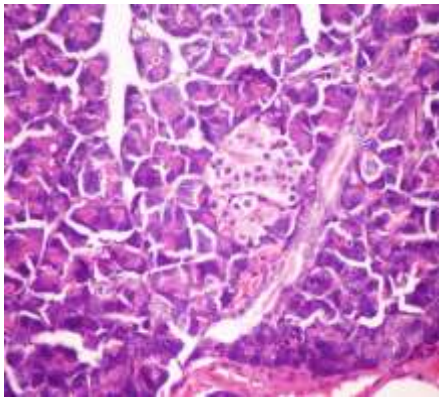

Photo (1): Photomicrograph of pancreas sections of rats from normal group showing no histological changes (H and E X400).

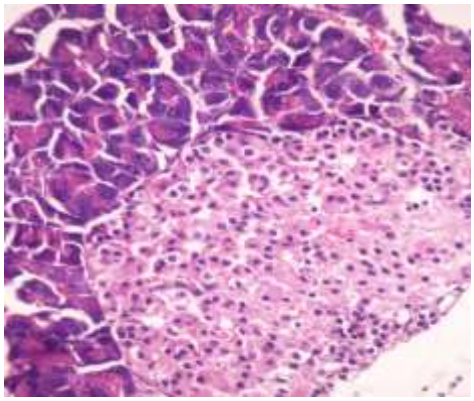

Photo (2): Photomicrograph of pancreas sections from obese-diabetic rats from positive control group showing hypertrophy and hyperplasia of β-cells of islets of langerhans associated with pyknosis of their nuclei **(**H and E X400).

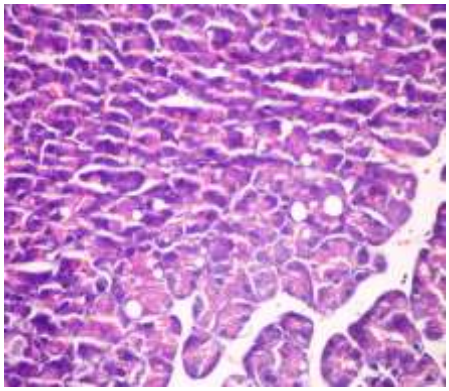

Photo (3): Photomicrograph of pancreas sections from obese-diabetic rats treated group with 5% chard leaves showing vaculations of acinar epithelial lining (H and E X 400).

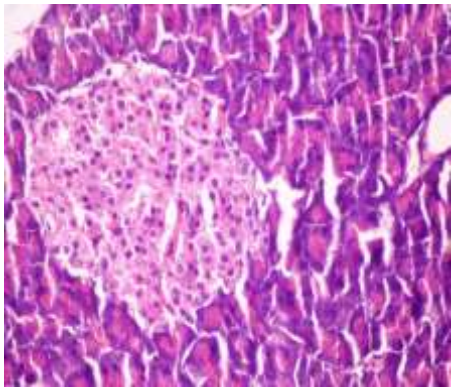

Photo (4): Photomicrograph of pancreas sections from obese-diabetic rats treated group with 7.5 and 10% chard leaves showing vaculations of acinar epithelial lining (H and E X 400).

2-Histopathological Examination of Liver:

Histopathological examination of liver sections of health rats from the negative group showed normal histological in liver structure as shown in **Photo 5.** In comparison to the negative control group, examined liver sections of obese-diabetic rats from the positive control group revealed fatty change of hepatocytes and congestion of hepatic sinusoid, vacuolization of hepatocytes, and necrosis of sporadic hepatocytes as shown in **Photos 6 .** This result agreed with the results of **Arkkila *et al*., (2001)** revealed that histological changes of liver sections of diabetic rats, which showed congestion of hepatic sinusoids, vacuolization of hepatocytes, and necrosis of sporadic hepatocytes as well as fatty changes of hepatocytes. This observation was agreed with **Rezq, (2011)** which showed that alloxan-induced diabetic rats have a congestion of the hepatic sinusoid, vacuolization of hepatocytes, and necrosis that was shown in sporadic hepatocytes. In previous studies, HFD-fed STZ-induced rat models were reported to have fatty liver and histopathological changes including lipid accumulation and lobular inflammation (**Guo *et al*., 2018, and Dwivedi and Jena, 2020**). Also, **Wickramasinghe *et al.*, (2024)** showed the major histopathological alteration observed in liver tissues of STZ-induced diabetic rats was hydropic degeneration of hepatocytes and mild lobular inflammation.

On the other hand examining liver sections of obese-diabetic rats from the treated group with added 5% of chard leaves to HFCD revealed fatty change of hepatocytes (**Photo 7**) in some sections. Likewise, examined liver sections of

obese-diabetic rats from the treated group with added 7.5% of chard leaves to HFCD revealed small vacuoles in the cytoplasm of hepatocytes as shown in **Photo 8**. Furthermore, examined liver sections of obese-diabetic rats from the treated group with added 10% of chard leaves to HFCD revealed small vacuoles in the cytoplasm of some hepatocytes (**Photo 9**).

Ozsoy-Sacan *et al*., (2004) concluded that the chard leaves extract has a protective effect on the liver in diabetes mellitus. The administration of chard extracts improved histopathological damage apart from necrosis and vacuolization. The antihyperglycemic effects and the prominent improvement in the tissues may be related to the flavonoid and saponin content of this plant. **Gezginci-Oktayoglu *et al*., (2014)** showing many hepatocytes have intense eosinophilic cytoplasm and pycnotic nuclei, rupturing the endothelium of central veins, sinusoidal dilatation and necrosis were determined in the liver of hyperglycemic animals. While the administration of chard extract improved histopathological damage apart from necrosis and vacuolization. They suggested that chard extract can improve liver damage in diabetic rats, potentially through antioxidant and anti-inflammatory mechanisms. **Helmy *et al*., (2024)** reported that the chard leaf powders displayed powerful positive hypoglycemic action compared to metformin, in addition to reversing the histological abnormalities in the liver and pancreas of diabetic rats to a state close to normal.

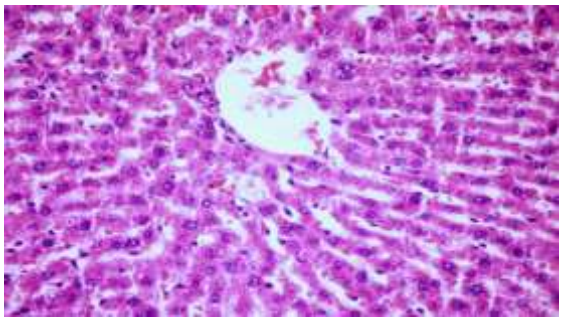

Photo (5): Photomicrograph of liver sections of health rats from the negative group showing normal histological structure of hepatic lobule (H and E x 400)

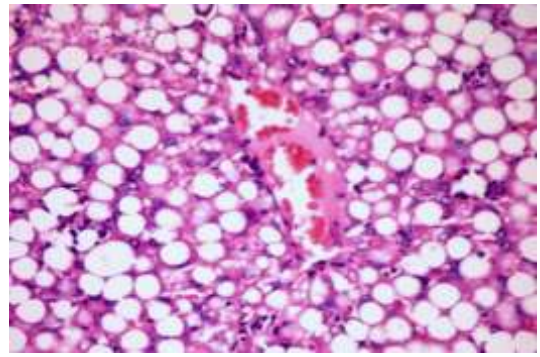

Photo (6): Photomicrograph of liver sections of obese-diabetic rats from the positive control group showing fatty change of hepatocytes (H and E x 400).

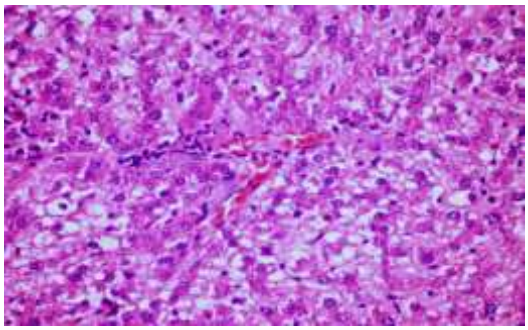

Photo (7): Photomicrograph of liver sections of obese-diabetic rats from the treated group with added 5% of chard leaves to HFCD showing fatty change of hepatocytes (H and E x 400).

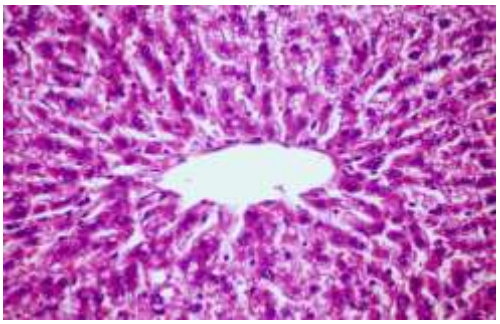

photo (8): Photomicrograph of liver sections of obese-diabetic rats from the treated group with added 7.5% of chard leaves to HFCD showing small vacuoles in the cytoplasm hepatocytes (H and E x 400).

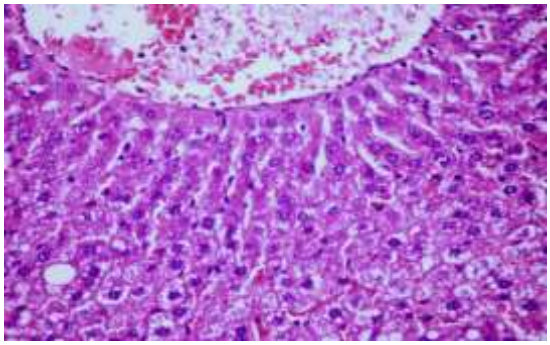

Photo (9): Photomicrograph of liver sections of obese-diabetic rats from the treated group with added 10% of chard leaves to HFCD showing small vacuoles in the cytoplasm of some hepatocytes (H and E x 400).

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فعالية اوراق السلق في التحكم في وزن الجسم ومستويات الجلوكوز في الدم في الفئران البدينة المصابة بالسكر

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المُستلخص العربى

تُعدّ زيادة الوزن والسمنة من أبرز الأمراض الناتجة عن أنماط الحياة الحديثة، والتي تؤدي إلى مشكلات صحية أخرى وتساهم في العديد من الأمراض المزمنة. مرض السكر هو مصطلح شامل يُطلق على اضطرابات التمثيل الغذائي المتنوعة، والتي تتمثل أعراضها الرئيسية في ارتفاع مستوى السكر في الدم. وقد تم إجراء هذه الدراسة للتحقق من تأثير أوراق السلق في التحكم في وزن الجسم وخفض مستوى السكر في الدم في الفئران البدينة المصابة بالسكر. تم استخدام ٣٥ فأرًا ذكرًا من فئران الألبينو. حيث تم إحداث السمنة في ٢٨ فأرًا عن طريق تغذيتهم على نظام غذائي مرتفع الدهون والسعرات الحرارية لمدة أربعة أسابيع. بعد ذلك، تم حقن الفئران المصابة بالسمنة بمادة الستربتوزوتوسين تحت الجلد بجرعة ٦٠ ملجم/كجم من وزن الجسم لإحداث الإصابة بمرض السكر. تم حقن مجموعة من الفئران الغير بدينة وغيرمصابة بمرض السكر (٧ فئران) بكمية مماثلة من محلول ملحي، وتم اعتبارها كمجموعة ضابطة سالبة. تم تقسيم الفئران البدينة المصابة بالسكر إلى ٤ مجموعات. المجموعة الثانية، وهي المجموعة المصابة غير المعالجة، وتم اعتبارها كمجموعة ضابطة مُوجبة. بينما تم علاج المجموعات الثلاث الأخرى المصابة بالسمنة والسكر بالتغذية على نظام غذائي عالي الدهون مضاف إليه ٥٪ و٧,٥٪ و١٠٪ من مسحوق أوراق السلق، بنسبة متناسبة مع النظام الغذائي. أظهر التقدير الكمي أن محتوى الفينولات الكلية كان ٤١,٠٠±٢,٠١ ملجم مكافئ حمض الجاليك/جم، ومحتوى الفلافونويدات الكلية كان 3.26±1.05 مكافئ كيرسيتين/جم. أظهرت النتائج أن الفئران المصابة بالسمنة والسكر غير المعالجة شهدت انخفاضً ملحوظً في كمية الطعام المأخوذ، و متوسط قيم مستويات الليبوبروتينات المرتفعة الكثافة، ونشاط إنزيمات مضادات الأكسدة(الجلوتاثون والسوبر أكسيد ديسميوتيز) في الدم. بينما لوحظ ارتفاع معنوى في متوسط متوسط القيم لكل من الوزن النهائي، والزيادة في وزن الجسم، نسبة الزيادة في وزن الجسم، وزن الدهون المخزنة بالجسم، مؤشر السمنة، ومستويات سكر الدم، هرموني الإنسولين واللبتين، الكوليسترول الكلي، الدهون الثلاثية، الدهون الكلية، والليبوبروتينات المنخفضة والمنخفضة جداً في الكثافة، المالون داي ألدهيد، ونشاط إنزيمات الكبد (الأسبارتات ترانس أمينيز ، ألانين ترانس أمينيز ،والألكين فوسفاتيز) في الدم وذلك مقارنةً بفئران المجموعة الضابطة السالبة.بالاضافه الى ذلك، أظهر الفحص النسيجي المرضي لبنكرياس الفئران البدينة المصابة بالسكر(المجموعة الضابطة الموجبة) تضخمًا وتكاثراً في خلايا بيتا في جزر لانجرهانز، مصحوبًا بتضخم أنويتها. كما أظهرت مقاطع الكبد تغيرات دهنية في خلايا الكبد، واحتقانًا في الجيوب الكبدية، وتجويفًا في خلايا الكبد، وثقوب في خلايا الكبد المتفرقة. ومن المثير للاهتمام أن الفئران المصابة بالسمنة والسكر من المجموعات المعالجة التي تغذت على نظام غذائي مدعم بأوراق السلق بمستويات ٥ و٧,٥ و١٠٪ أظهرت تحسن في جميع المعايير البيولوجية والكيميائية الحيوية التي تم اختبارها، بالإضافة إلى الدراسة النسيجية للبنكرياس والكبد. ازدادت فعالية أوراق السلق في علاج الفئران المصابة بالسمنة والسكر بشكل ملحوظ مع زيادة مستويات أوراق السلق مع نظام غذائي عالى الدهون والسعرات الحرارية. وأخيرًا، أوضحت الدراسة أن أوراق السلق لها تأثيرات مفيدة على الفئران المصابة بالسمنة والسكر التي تتغذى على نظام غذائي عالى الدهون والسعرات الحرارية، من خلال تقليل زيادة الوزن النسبية، والأنسجة الدهنية، دهون الدم، وتحسين وظائف الكبد، وتقليل أكسدة الدهون.

الكلمات المفتاحية: السلق، السمنة، داء السكري، النظام الغذائي عالي الدهون ، الستربتوزوتوسين، إنزيمات مضادة للأكسدة.