Research Article

Cranberry Extract; as a Promising Functional Food to Regulate SREBP1/PPAR–α/CPT-1/ACO Signaling Pathways in HFD-Induced Obesity in Rats

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Abstract | The correlation between obesity and metabolic disorders like Type 2 diabetes, cardiovascular disease, and several types of cancer has made obesity a challenge for world health. The current study's objective was to assess the antioxidant capacity of cranberry extract in high-fat diet-induced obesity. For eight weeks, 36 white albino rats weighing 197±10 g were selected at random to five groups: Group I was administered a regular diet; Group II was fed a high-fat diet; Group III was given a high-fat diet plus cranberry extract (75 mg/kg); Group IV was fed a high-fat diet with cranberry extract (150 mg/kg); and Group V was fed a high-fat diet plus metformin (500 mg/kg). The lipid profile was determined using blood samples, and liver tissue samples were used to determine glucose, insulin, leptin, TC, TG, HDL-C, ALT, AST, MDA, TNF-α, PCO. The HFD administrated rats resulted in increased total body weight, glucose, insulin, leptin, cholesterol, triglycerides, MDA, ALT, AST, TNF- α and PCO levels, as well as SREBP1 gene expression, according to the findings. However, our findings revealed a significant reduction in HDL-C, GSH, CAT and PON 1 enzyme levels, the expression of the PPAR– α, CPT I and ACO gene in HFD treated rats. Also, administration of cranberry extract and metformin significantly normalized body weight, blood glucose, insulin, leptin, total cholesterol, triglycerides, HDL-C, TBARS, GSH, CAT, ALT, AST, TNF- and PCO and PON 1 as well as SREBP1, PPAR– α, CPT I and ACO levels. The biochemical and PCR results are supported by electrophoretic patterns and histological evidence. Conclusions: The findings imply that cranberry extract could be used as a new pharmaceutical drug in the treatment of obesity.

Keywords | Cranberry extract, Insulin, Leptin, Total cholesterol, Paraoxonase1

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INTRODUCTION

Hyperlipidemia, T2D, and cardiovascular disease are all conditions that affect the body's lipid levels and related to obesity and insulin resistance (Fried et al., 2008; Yalniz et al., 2007; Pagotto et al., 2008; Lavie et al., 2008). SREBP-1c gene transcription in mice and humans was regulated by the effects of insulin and the presence of cel-

lular cholesterol (Borik and Hussein, 2021; Boshra and Hussein, 2016; Hussein et al., 2017).

PPAR is involved in glucose and lipid homeostasis and can be found in a variety of tissues (Elgizawy et al., 2019; 2021). Eicosanoids, fatty acids, and derivatives of fatty acid are all endogenous PPAR ligands (Mosaad et al., 2016). It is a switch that regulates mesenchymal stem cell development into adipocytes (El Gizawy et al., 2021b).

CPTI is a protein catalysis the rate-limiting phase of fatty acid oxidation, as well as has identified as a possible a new objective treatment of diabetes and obesity (Shehata et al., 2015). Hepatocyte dysfunction is caused by administration of HFD and generation of reactive oxygen species, which inhibit ACOX1 (Ghorab et al., 2010; Hussein, 2013). Natural anti-oxidants acquired from many alternative medical systems have been shown in multiple studies to exhibit a wide range of biological activity (Abdel-Gawad et al., 2003; Hussein, 2012). Several antioxidant-rich substitutes have been utilised to decrease oxidative stress in animal models (Fayed et al., 2022).

Several plant extracts have been showed to help reduce organ toxicity (Aherne and O'Brien, 2002; Mullen et al., 2007; Vvedenskaya et al., 2004). One of the best source of flavanols is cranberry fruits (Manach et al., 2004; Viskelis et al., 2009). The most common flavanol in cranberries is quercetin, as 3-o-galactoside, anthocyanin, myricetin and kaempferol (Soliman et al., 2022).

There are no publications on the antioxidant properties of cranberry extract in the prevention of HFD-induced obesity in rats. As part of our ongoing interest in the pharmacological and medical value of natural products (Abdel Maksoud et al., 2019; Fayed et al., 2022; Borik and Hussein, 2022), This study was created to assess the anti-diabetic and antioxidant effects of cranberry extract against obesity caused by the HFD.in rats.

MATERIALS AND METHODS

Chemicals:

- Virgin Extracts (TM) in China provided Cranberry extract.
- Metformin (98%), paracetamol (99%) and silymarin (98%) were purchased from (Sigma Aldrich, Germany).

Animals

Criteria for this experiment were developed by the animal committee of the National Cancer Institute, Cairo University. At the beginning of experiment, the adult rats weighing approximately 193±8 g. However, after 10 days animal adaptation, rats weighing approximately 197±10 g. They were housed in cages with an air conditioning system, kept at a temperature of 22 °C, 60 % relative humidity, and an 8:00–20:00 light cycle. During the period of acclimation, each animal was given a normal diet as needed. Table 2 explains the nutritional components of the regular diet and high-fat diet (Assinewe et al., 2003).

Table 2: Nutritional components of diet

Table 1: Treatment group descriptions

Groups	Treatment Description	Treatment Description
1	Regular diet (RD)	For an 8-week period, they received a normal diet
2	High- fat diets (HFD)	For an 8-week period, given a high-fat diet
3	HFD+ Cranberry extract (75 mg / kgbw)	Over an 8-week period, rats were administered a high-fat diet with cranberry extract (Fayed et al., 2022).
4	HFD+ Cranberry extract (150 mg / kgbw)	Rats were given a high-fat diet and receiving one dosage of cranberry extract orally every day (Fayed et al., 2022).
5	HFD plus metformin (500 mg/kgbw)	During an 8-week timeframe, rats were fed a HFD with one dose metformin orally (Fayed et al., 2022).

Biochemical tests

Body weights were measured biweekly. At the end of the study, blood samples were collected in heparinized tube, centrifugate, and the obtained plasma was used to estimated glucose colomerically (Troisi et al., 2003).

An ultra-sensitive rat insulin ELISA kit was used to determine the levels of plasma insulin (Alpco Diagnostics) (Finlay and Dillard, 2007). Using commercially available kits, plasma TG, TC, and HDL-C were measured (Asan and Youngdong Pharmaceutical Co., Korea) (Allain et al., 1974; Fossati and Prencipe, 1982; Burnstein et al., 1970).

Also, the liver was removed directly used for estimation of hepatic TBARS and GSH. In phosphate buffer saline (Ph 7.4, 1 mL), liver tissue (100 mg) was homogenized. The homogenate (0.2 ml) was extracted using a 2:1 chloroform-to-methanol ratio, and the extract was then concentrated with nitrogen stream. For TBARS estimation, the residue was employed (Tsikas, 2017). To estimate the live GSH, another piece of the tissues was homogenized with phosphate buffer saline (Owen and Butterfield, 2010).

Diet content	Regular diet	High fat diet
Wheat flour (%)	22.5	22.5
Soybeen powder (%)	25	25
Essential Fatty Acids (%)	0.6	0.6
Vitamins/kg. diet		
A	0.6 mg	0.6 mg
D	1000 IU	1000 IU
E	35 mg	35 mg
Niacin	20 mg	20 mg
Pantothenic acid	8 mg/kg	8 mg/kg
Riboflavin	0.8 mg	0.8 mg
Thiamin	4 mg	4 mg
Vit.B6	50 µg	50 µg
Vit.B12	7 mg	7 mg
Minerals / kg. diet		
Calcium	5 g	5 g
Phosphorus	4 g	4 g
Fluoride	1 mg	1 mg
Iodine	0.15 mg	0.15 mg
Chloride	5 mg	5 mg
Iron	35 mg	35 mg
Copper	5 mg	5 mg
Magnesium	800 mg	800 mg
Potassium	35 mg	35 mg
Manganese	50 mg	50 mg
Sulfur	3 mg	3 mg
Fat/kg	-	200 g
Cholesterol	-	1% (w/w)

Table 3: Primer sequences for real-time PCR

Gene	Sequence of primers	Amplicon size	Annealing temperature	Accession number
SREBP1	F:5′-TCTGCCTTGATGAAGTGTGG-3′ R:5′-AGCAGCCCCTAGAACAAACA-3′	80	55°	NM001005291
ACO	F:5′-GTTAGCAACTGGGATGATATGG-3′ R:5′-AGCACCAATCGTGATGACTTG -3′	37	57°	U68215
CPT-1	F:5′-AAGGAATGCAGGTCCACATC-3′ R:5′-CCAGGCTACAGTGGGACATT-3′.	78	57°	XM_218625
PPAR–α	F:5′-TCGAGGAAGGCACTACACCT-3′ R:5′-TCTTCCCAAAGCTCCTTCAA-3’	337	59°	NM_005036
β-actin (internal control for qRT-PCR)	F:5′-GATTACTGCTCTGGCTCCTGC-3′ R:5′-GACTCATCGTACTCCTGCTTGC-3	100	56.5°	AF110103.1

CAT was measured using the Sinha method, which relied on the production of chromic acetate from dichromate and glacial acetic acid in the presence of hydrogen peroxide. The produced chromic acetate was measured calorimetrically at 570 nm, and one enzyme unit was defined as the amount of enzyme that catalyzed the oxidation of 1 mole H_2O_2 per minute (Hadwan, 2018).

A PON1 activity of 1 U/mg protein was defined as 1 mol p-nitrophenol generated per minute per mg protein. PON1 activity towards paraoxon was measured by evaluating the initial rate of substrate hydrolysis to p-nitrophenol, whose

absorbance was monitored at 405 nm in the test mixture (Jeelani et al., 2019).

In order to create protein hydrazones, which were then spectrophotometrically quantified, dinitrophenylhydrazine reacts with protein carbonyls to generate a Schiff base. After precipitating the protein with an equivalent amount of 1% trichloroacetic acid, it was resuspended in 10 mmol/L DNPH + 2N HCl, or in 2N HCl as a control blank. Washing with 1:1 ethanol-ethyl acetate and dissolved in 6 mol/L guanidine (Levine et al., 1990). The carbonyl group was identified by measuring the absorbance at 370 nm. The carbonyl content (nmol/mg protein) was estimated according to the method of (Reznick et al., 1994).

Evaluation of plasma ALT, AST and hepatic TNF-α were done by the methods described by Reitman and Frankel (1957), Beyaert and Fiers (1998), respectively.

qRT-PCR

Rat liver was used to extract the total RNA, and using Sepasol-RNA1Super and the manufacturer's instructions, portions (10–15 g) of the obtained RNA were subjected to real-time PCR analysis. Measurements of RT-PCR gene expression steps have been made. PPAR- –α, CPT1, ACO, and SREBP1 expression levels were measured. The test mixture for the single-plex reaction was 50 ml. The reaction was conducted under the following conditions: pre-incubation at 50 °C for 2 minutes, then 10 min. of 40 cycles of 95 °C in 15 s. and 60 °C in 1 min. The following table provided an illustration of the primer sequences (3).

Histological Examination

A sample of liver tissue was obtained and fixed in neutral buffered formalin (10%) before being dehydrated in ethyl alcohol (50–100%), cleaned in xylene (3 times), embedded in molten paraffin wax, and then cut into blocks with a rotary microtome to a thickness of 5–6 microns. The paraffin sections were stained using a H&E stain as methods described by Bancroft and Steven (1983) and exanimated using (Olympus, Münster, Germany) light microscope. Photomicrograph of the liver tissue was taken at magnification (x 400).

Statistical analysis

Data were expressed as means ± SD. ANOVA with post hoc Bonferroni correction was used to evaluate all data using the SPSS/20 programme. The significant differences between the two groups of overweight, class I and II obese diabetic patients, and control participants were compared using a student's t test. Statistical significance was defined at P less than 0.05 (Snedecor and Cochran, 1969).

RESULTS AND DISCUSSION

Obesity has been associated with heart disease, diabetes disease, irritable bowel syndrome, nonalcoholic fatty liver disease, pancreatitis, cancer, and other conditions (Genser et al., 2016; Mandviwala et a., 2016). Nutritional supplement based on therapies are quite popular, especially when it comes to obesity and body composition. Obesity treatment has used inhibition of dietary of fat digestion and absorption of the diet as a target (Li et al., 2016).

Each bimonthly, the body weight was recorded. As the rats developed over the 8-week study, their body weights in the regular-fed rats steadily increased (Table 4). When compared to the control group, the rats' weight after 8 weeks of HFD feeding considerably increased from the start of the feeding period to the end. Rats receiving cranberry extract (75 and 150 mg) and metformin (500 mg) the body weight was reduced to 17.3, 21.3 and 22.11%, respectively, relative to HFD-fed rats of rats after 8 weeks ($p< 0.05$), respectively.

The anti-obesity properties of cranberry, Obese diabetic rats fed a HFD as a model of obese T2D were used to test the extract. At the age of eight weeks, Obesity and T2D occur in rats fed a HFD, and these rats are often used in obesity and diabetes studies (Estadella et al., 2004).

After eight weeks, plasma levels of glucose, leptin, and insulin were measured and compared across groups in Table 5. When compared to a group of rats on a regular diet, rats fed the HFD had significantly higher levels of insulin, leptin, and glucose (1.93, 4.3, and 1.85 folds higher, respectively). After 8 weeks, the insulin, leptin, and glucose levels in the rats receiving cranberry extract (75 and 150 mg/kg) and metformin (500 mg/kg) were significantly lower than those in the HFD-fed rats ($p < 0.05$).

Cranberry extract was discovered to have anti-obesity characteristics, as it significantly reduced body weight growth. Severe type II diabetes was established in the high-fat diet group, which had significantly higher plasma glucose and insulin levels than the conventional diet group. Glucose and vg levels in the blood have increased as a result of these changes. Cranberry extract was found to lower insulin levels. We can speculate activation of cell electrical activity by cranberry extract can increase insulin release (Zhang et al., 2019).

With the exception of HDL-C, plasma TC and TG levels in high fat diet-fed rats were noticeably higher than those in normal diet-fed rats (Table 6). When compared to regular diet group, the plasma TG and TC levels of rats fed HFD were significantly higher (1.8 and 1.45 folds, respec

Table 4: Changes in body weight of control and experimental groups of rats

No.	Groups	Number of weeks Body weight of rats(g)				
		0	2	4	6	8
(I)	Regular diet (RD)	206.7 ±8.60^{Aa}	219.8 ±9.80^{Ba}	226.5 ±11.45^{Ba}	233.8 ±10.00^{Ca}	242.67 ±14.7^{Da}
(II)	High-fat diet (HFD)	210.76 ±14.00^{Aa}	237.60 ±10.50^{Bb}	260.50 ±17.60^{Cc}	282.70 ±13.50^{Dc}	310.6 ±15.60^{Ed}
(III)	HFD+ Cranberry extract (75 mg/kg.b.w.)	209.4 ±16.00^{Aa}	214.6 ±11.50^{Aa}	234.7 ±14.00^{Bc}	246.90 ± 12.25^{Cb}	256.80 ±11.67^{Dc}
(IV)	HFD+ Cranberry extract (150 mg/kg.b.w.)	204.6 ± 10.76^{Aa}	215.40 ± 18.90^{Aa}	225.90 ±11.20^{Ba}	236.60 ±16.80^{Ca}	244.30 ±14.00^{Da}
(V)	HFD + Metformin (500 mg/kg.b.w.)	210.8 ± 12.30^{Aa}	212.70 ± 12.70^{ABa}	220.40 ± 17.80^{Ba}	235.80 ± 16.00^{Ca}	241.90 ± 12.50^{Ca}

Body weight of rats consuming regular diet, high fat diet, high fat diet plus Cranberry extract (75 and 150 mg/kg.b.w.) and Metformin (500 mg/kg.b.w.) during the 8-week period. Values are given as mean ± SD significantly different at P ≤ 0.05 for groups of eight animals each. Small letters are used for comparison between the means within the column. Capital letters are used to compare means within the row.

Table 5: Effect of Cranberry extract and metformin on plasma insulin, leptin and glucose HFD-induced obesity in rats

No.	Groups	Plasma insulin (ng/dl)	Plasma leptin (ng/dl)	Plasma glucose (mg/dl)
(I)	Regular diet (RD)	1.09 ± 0.17^{a}	1.71 ± 0.12^{a}	101.92 ± 7.40^{a}
(II)	High-fat diet (HFD)	2.74 ± 0.42^{c}	7.42 ± 0.54^{d}	189.09 ± 7.33^{c}
(III)	HFD+ Cranberry extract (75 mg/kg.b.w.)	2.01 ± 0.09^{b}	4.00 ± 0.60^{c}	153.03 ± 5.15^{b}
(IV)	HFD+ Cranberry extract (150 mg/kg.b.w.)	1.23 ±3.38^{a}	2.58 ±0.40^{b}	112.17 ±8.68^{a}
(V)	HFD + Metformin (500 mg/kg.b.w.)	1.22 ±0.13^{a}	2.90 ±0.25^{b}	103.79 ±8.84^{a}

Values represent the mean ± SE (n=6).
Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at P ≤ 0.05.

Table 6: Effect of Cranberry extract and Metformin on plasma cholesterol, triglycerides and cholesterol-high density lipoprotein (HDL) HFD-induced obesity in rats

No.	Groups	Plasma cholesterol (mg/dl)	Plasma triglycerides (mg/dl)	Plasma HDL (mg/dl)
(I)	Regular diet (RD)	113.22 ± 9.31^{a}	71.85 ± 6.33^{a}	39.62 ± 2.70^{a}
(II)	High-fat diet (HFD)	172.83 ± 7.31^{c}	104.59 ± 6.86^{c}	27.95 ± 3.76^{c}
(III)	HFD+ Cranberry extract (75 mg/kg.b.w.)	139.72 ± 6.58^{b}	93.90 ± 6.39^{b}	33.72 ± 2.42^{b}
(IV)	HFD+ Cranberry extract (150 mg/kg.b.w.)	109.90 ±10.04^{a}	71.40 ±5.22^{a}	40.12 ±2.79^{a}
(V)	HFD + Metformin (500 mg/kg.b.w.)	115.62 ±8.89^{a}	69.14 ± 3.97^{b}	32.53 ±4.29^{b}

Values represent the mean ± SE (n=6). Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at P ≤ 0.05.

Table 7: Effect of Cranberry extract and Metformin on hepatic thiobarbaturic acid reactive substances (TBARS), reduced glutathione (GSH), catalase (CAT) and paraoxonase1 (PON1) HFD-induced obesity in rats

No.	Groups	TBARS (nmol/g wet tissue)	GSH (mg/ g tissue)	CAT (µmol H_2O_2 consumed/ mg protein/min)	PON 1 (U/mg protein/ min)
(I)	Regular diet (RD)	1.50 ± 0.15[a]	59.09 ± 5.68 [a]	24.26 ± 3.08[a]	247.99 ± 9.70 [a]
(II)	High-fat diet (HFD)	3.86 ± 0.59[b]	30.35 ± 6.61 [c]	11.96 ± 1.78[c]	145.55 ± 7.60[e]
(III)	HFD+ Cranberry extract (75 mg/kg.b.w.)	1.45 ± 0.25[a]	42.63 ± 4.61 [b]	17.45 ± 1.93 [b]	212.97 ± 7.07[d]
(IV)	HFD+ Cranberry extract (150 mg/kg.b.w.)	1.42 ±0.17 [a]	52.66 ±3.64[a]	24.21 ±3.04 [a]	234.10 ±8.78[b]
(V)	HFD + Metformin (500 mg/ kg.b.w.)	1.54 ±0.16[b]	40.32 ±5.21 [b]	15.26 ±3.31[b]	182.99 ±10.14[c]

Values represent the mean ± SE (n=6). Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at P ≤ 0.05.

Table 8: Effect of Cranberry extract and Metformin on plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as liver tumor necrosis factor- alpha (TNF-α) and protein carbonyl (PCO) in HFD-induced obesity in rats

No.	Groups	Plasma ALT (U/L)	Plasma AST (U/L)	Hepatic TNF-α (pg/mg tissue)	Hepatic PCO (nmol/g wet tissue)
(I)	Regular diet (RD)	41.24 ± 3.47 [a]	17.36 ± 2.96 [a]	9.38 ± 0.75 [a]	13.84 ± 2.90[a]
(II)	High-fat diet (HFD)	78.32 ± 4.84 [c]	47.19 ± 4.62 [c]	14.48 ± 1.09 [b]	22.12 ± 2.04[c]
(III)	HFD+ Cranberry extract (75 mg/kg.b.w.)	58.00 ± 8.77[b]	30.06 ± 5.08 [b]	11.39 ± 1.41 [a]	15.98 ± 1.88[ab]
(IV)	HFD+ Cranberry extract (150 mg/kg.b.w.)	44.00 ±6.65[a]	21.78 ±4.62 [a]	9.21 ±0.98 [a]	14.76 ±3.38[ab]
(V)	HFD + Metformin (500 mg/ kg.b.w.)	57.79 ±4.71[b]	33.47 ±4.76 [b]	10.50 ±1.53 [a]	16.26 ±1.88[b]

Values represent the mean ± SE (n=6). Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at P ≤ 0.05.

tively, while HDL-C levels were significantly lower (1.42-fold) (p<0.05). Administration of cranberry extract (75 and 150 mg) and metformin (500 mg) significantly decreased the levels of TG and TC as well as increased HDL-c levels as compared to the HFD fed rats (p< 0.05).

Additionally, fat accumulation was reduced, indicating that the substances examined suppressed TG, TC, and HDL-C. Because cholesterol deposition in atherosclerotic plaques is thought to be caused by lipoprotein fractions containing apo-B, the lipoprotein fraction accounts for most of the drop in plasma cholesterol (Istek and Gurbuz, 2017). This alteration could be related to a decrease in cholesterol, and triacylglycerols would be beneficial in therapeutic settings. Extract improved hypercholesterolemia caused by a high-fat diet. Furthermore, dietary treatment with cranberry extract reduced plasma cholesterol and triacylglycerols, in liver rats (Demori et al., 2007). Additionally, alterations in the activity of HMG-CoA reductase and cholesterol 7-hydroxylase are cholesterol metabolism enzymes may be responsible for the reduced plasma cholesterol levels of rats given HFD. Our study has been proposed that a cholesterol-lowering effect of cranberry extract could be achieved through stimulating hepatic cholesterol-7-hydroxylase activity. However, in T2D mice, polyphenol therapy reduced the HMG-CoA reductase activity in the liver (Moya et al., 2007). Cranberry is best source for quercetin, delphinidin, malvidin, petunidin 3-glucosides, 3-arabinosides, and 3-galactosides (Rozenberg and Aviram, 2006; Fayed et al., 2022). Also, the effect of eating blueberries on obese people's body weight and metabolism during a 12-week nutrition therapy (Hafize et al., 2007). According to the findings, blueberries-supplemented group shed a significant amount of weight and body fat. When compared to baseline and placebo control, the blueberry group's TC, LDL-C, insulin, insulin resistance, and uric acid levels im-

proved significantly. blueberry-supplemented group had a 2.5 to 3-fold reduced BMI at the end of 12-week, which a significant health benefit (Wu et al., 2018).

Hepatic TBARs levels in HFD fed rats were dramatically increased to 2.57-fold as well as significantly decreased of GSH, CAT and PON1 to 1.96-, 2.03 and 1.70-folds with compared to regular diet fed rats (Table 7) ($p< 0.05$). In comparison to the HFD-fed rats, the administration of cranberry extract (75 and 150 mg) plus metformin (500 mg) significantly reduced TBARs and elevated GSH, CAT, and PON 1 levels ($p< 0.05$).

The high-fat diet control group had considerably higher levels of liver TBARS., although the level of GSH, CAT, and PON1 were significantly lower. By creating too many reactive oxygen species, hyperglycemia induces oxidative stress (ROS). These ROS harm organs oxidative damage is common in organs. Oxidative damage is common in organs including the heart, liver and lung often more severe in diabetic patients (Hussein et al., 2021). Cholesterol and triacylglycerols were shown to normalize the antioxidant enzyme activity of the liver when rats were fed a HFD. Supplementing these enzymes in liver with the compounds tested may have helped maintain antioxidant balance in rats on HFD. According to multiple studies, flavonoids lowers lipid peroxidation and boosts the activities of increase antioxidants activity including GSH, CAT, and PON1, in a variety of malignant diseases of diverse physiological organs (Botham et al., 2013; Cheng et al., 2021). In compared to the control group, cranberry extract significantly decreased liver MDA levels and enhanced CAT and PON1 activities. Increased lipid peroxidation induces enzyme inactivation due to MDA crosslinking, resulting in a rise in Radicals such as superoxide, H_2O_2, and hydroxyl radicals have the ability to induce lipid peroxidation (Nakajima et al., 2002).

Furthermore, we discovered MDA and PCO levels have a negative connection. in liver tissues as well as CAT and PON 1 enzyme activity. This finding with supported by the idea that obesity causes antioxidant enzymes and proteins to be inactivated by large amounts of lipid peroxidation. The decrease in antioxidant enzyme could be explained by the quick consumption and exhaustion of this enzyme's storage capacity to fight free radicals created during the development of obesity. Due to insufficient lecithin cholesterol acyl transferase (LCAT) activity, PON-1 activity may be reduced as a result of decreased HDL synthesis and/or secretion. PON-1 has been demonstrated to become inactive under oxidative stress due to the redox regulatory process, which produces a mixed disulfide from a protein thiol and oxidized glutathione (Beites et al., 2021). Table 8 shows a significant increase of ALT, AST, TNF-α and PCO levels by 1.9-, 2.72, 1.54 and 1.60-folds in HFD fed rats as compared to the regular diet fed rats ($p< 0.05$). Administration of cranberry extract (75 and 150 mg) and metformin (500 mg) significantly decreased of ALT, AST, TNF-α and PCO levels as compared to the HFD fed rats ($p< 0.05$).

Cranberry extract supplementation with cranberry extract therapy significantly lowered TG levels and increased GSH activity in HFD-treated rats. The expression of ALT, AST, TNF-, and PCO was drastically reduced in the cranberry extract therapy group. Glycerophospholipid and glutathione metabolism, as well as the insulin signalling system, were all affected by cranberry extract treatment intake. It's assumed that free radical-induced oxidative damage targets cellular proteins.

Figure 1 declares a significant ($P < 0.05$) decreased in liver PPAR–α, CPT1 and ACO genes expression, in HFD-fed rats as compared with the regular diet-fed rats. Administration of cranberry extract (75 and 150 mg) and metformin (500 mg), they show a significant increase in liver PPAR–α, CPT1 and ACO genes expression as compare to HFD-fed rats ($P < 0.05$). Also, in HFD fed rats, liver SREBP1 genes expression declared significant increase, when compared with the regular diet-fed rats. Also, treatment of rats with cranberry extract (75 and 150 mg/kg.b.w.) and metformin (500 mg/kg.b.w.) reduced the level of liver SREBP1 genes expression significantly comparing to HFD-fed rats.

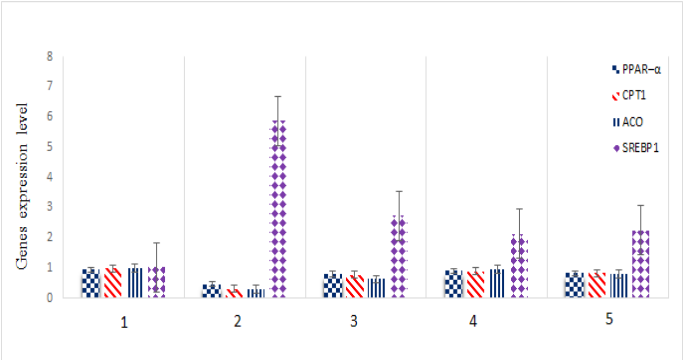

Figure 1: Effect of cranberry extract and metformin on liver PPAR–α, CPT1, ACO and SREBP1 gene expression in HFD-induced obesity in rats. Representative bar diagram of three independent experiments is presented. Group II: regular diet (RD) (1), Group II: high-fat diet (HFD) (2); Group III: Was administrate HFD+ cranberry extract (75 mg/kg.b.w.) (3); Group IV: Was administrate HFD+ cranberry extract (150 mg/kg.b.w.) (4), Group V: Was administrate HFD+ metformin (500 mg/kg.b.w.) (5).

The damage that ROS causes to cellular proteins may be the reason for the increase in PCO obesity. The accumulation of oxidized proteins in the cell may put the cell's

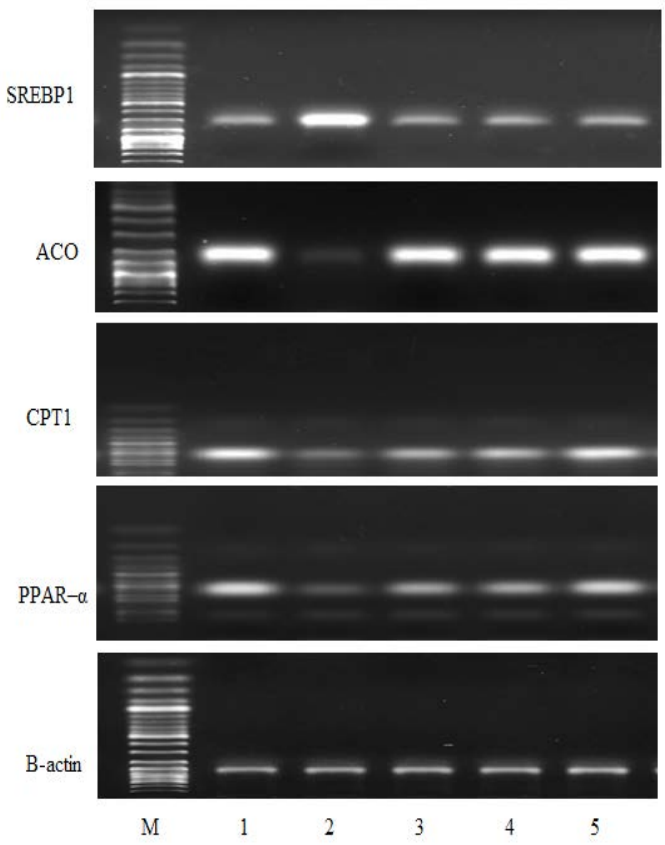

Figure 2: An agarose gel electrophoresis shows PCR products of liver PPAR–α, CPT1, ACO and SREBP1 gene expression in HFD-induced obesity in rats. M: DNA marker with 100bp. Group II: regular diet (RD) (1), Group II: high-fat diet (HFD) (2); Group III: Was administrate HFD+ cranberry extract (75 mg/kg.b.w.) (3); Group IV: Was administrate HFD+ cranberry extract (150 mg/kg.b.w.) (4), Group V: Was administrate HFD+ metformin (500 mg/kg.b.w.) (5).

capacity to function in jeopardy. Due to its relatively early production, higher stability and dependability, and longer useful life, PCO as a marker to evaluate damaged proteins may have certain advantages over other markers (Kohjima et al., 2008) Cranberry extract administration was found to attenuate inflammation and oxidative damage because of a high-fat diet increasing consumption of energy normalizing ALT, AST, TNF-α, and PCO levels (Kammoun et al., 2009).

PPAR–α regulates a variety of signalling pathways, glucose transport, liver lipid metabolism, and fatty acid synthesis by modulating SREBP1c transcription (Kang et al., 2013; Kuo et al., 2012). PPAR activation regulates the expression of the CPT1 and ACO genes, and CPT1 is involved in fatty acid oxidation (Messner et al., 2013). In our research, cranberry extract increased PPAR activity in a dose-dependent manner. HFD feeding, on the other hand, decreased the expression of CPT1 produced by cranberry extract, indicating a role for PPAR–α the regulation of CPT1. Furthermore, treatment of cranberry extract significantly inhibited SREBP1c gene expression, implying a role for PPAR-α lipid content. As a result, evidence for the role of CPT1 and ACO in fatty acid oxidation was presented by increased of the PPAR-α gene by cranberry extract (Zhu et al., 2014).

Also, lipoperoxidation is reduced, antioxidant status is improved, and the expressions of PPAR–α, CPT1and ACO are elevated in the liver of cranberry treated rats. stimulation of PPAR signaling and enhancement of mitochondrial function may be responsible for the positive effects of cranberry extract on steatosis and inflammation. Flavonoids can minimize induced lipid buildup by down-regulating proteins that cause lipogenesis up-regulating the manifestation of genes related to oxidation of fatty acids like PPAR-α, according to in vitro studies. Furthermore, this polyphenol appears to protect hepatocytes from lipoapoptosis (Aguirre et al., 2014), In cells treated with stearic acid, iron increased oxidative stress and insulin resistance, likely through lowering levels of phosphorylated JNK (Aoun et al., 2010; Panchal et al., 2013).

Histopathological examination of hepatocytes was intact with a clear outline, and the Diss cavity space was normal and arranged hepatic lobules around central veins (CV), normal sinusoids (S) in regular diet group (RD) (Figure 3a).

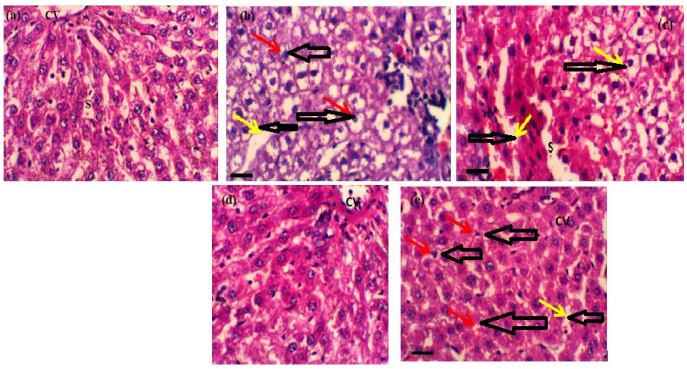

Figure 3: Sections stained with hematoxylin and eosin (H&E; 400 X) histological examination of rats liver of different groups compared to control group. Group II: regular diet (RD) (a), Group II: high-fat diet (HFD) (b); Group III: Was administrate HFD+ cranberry extract (75 mg/kg.b.w.) (c); Group IV: Was administrate HFD+ cranberry extract (150 mg/kg.b.w.) (d), Group V: Was administrate HFD+ metformin (500 mg/kg.b.w.) (e).

Moreover, microscopic pictures of H&E-stained liver sections (C, D) showing: the crowding of hepatocyte nuclei to the edge of the cell due to accumulation of lipid droplets. also, had unclear hepatocyte outlines (red arrows), and the Diss cavity of liver tissues disappeared. Dilated sinusoids

(yellow arrows). Many lipid droplets and cytoplasmic vacuoles were observed in the liver (Figure 3b).

Also, histological pictures of stained liver sections (E, F) show partially disrupted hepatic parenchyma due to decreased severity of ballooned hepatocytes degeneration and congestion (yellow arrows) also decrease congestion in the Central vein (CV) and inflammatory cell infiltration with still dilated sinusoids (S) (Figure 3c). Histological examination of stained liver sections (G, H) showed a completed regenerated hepatocytes nearly normal organization of hepatic parenchyma and radially arranged hepatic lobules around central veins (Figure 3d). Histological pictures of stained liver sections (I, J) showed mild regenerated hepatocytes around central veins central vein (Figure 3e).

Therefore, a HFD inhibits endogenous antioxidant defense systems, according to the current study, which looked at the effects of feeding cranberry extract with high fat meal for the lipid profile as well as antioxidant enzymes in liver. In hypolipidemic rats, the cranberry extract which had a significant lipid lowering effect, was also shown to considerably improve antioxidant system, as evidenced by the replenishment of depleted antioxidant molecules and a decrease in antioxidant enzyme activity. To the best of our knowledge. According to experts, Cranberry extract's anti-obesity effects in obese diabetic rats on a high-fat diet have never been documented., and this study could be the first of its kind.

CONCLUSIONS AND RECOMMENDATIONS

Cranberry extract exhibits a powerful anti-obesity and antioxidant effect against HFD-produced obesity in rats by modulating glucose, lipid profile, insulin, and oxidative stress indicators, according to this study. Furthermore, CPT1 and ACO are hypothesized to be involved in the anti-obesity effects of cranberry extract by activating PPAR– gene expression and down-regulating SREBP1 gene expression... These findings could offer molecular support for the use of cranberry extract as a treatment for obesity-related diseases. Overall, our findings indicated that cranberry extract could be a viable new anti-obesity and antidiabetic treatment option.

Abbreviation

Total cholesterol (TC), triglycerides (TG), High density lipoprotein-cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), tumor necrosis factor- alpha (TNF-α), protein carbonyl (PCO), reduced glutathione (GSH), catalase (CAT), paraoxonase1 (PON1), sterol regulatory element-binding protein 1 (SREBP1), peroxisome proliferator-activated receptor alpha (PPAR–α gene expression, carnitine palmitoyl transferase I (CPT I) and acyl-CoA oxidase (ACO) gene expression.

Significance Statement

In this investigation, it is shown that cranberry extract has hypolipidemic and anti-diabetic properties that may be useful in the treatment of obesity. With the aid of this study, the researcher will be able to examine crucial areas that many other researchers were unable to investigate about the evaluation of cranberry extract as a potential novel agent in the treatment of hyperlipidemia. As a result, a novel hypothesis explaining the relationship between obesity and CPT1, SREBP1, and ACO signalling may be developed.

DECLARATIONS

Acceptance of the ethics and consent to participate. Data collection received ethical approval from the Faculty of Applied Medical Sciences' Research Ethics Committee at October 6 University in Egypt (No. 20190715).

CONFLICT OF INTEREST

There are no financial or other conflicts of interest, according to the authors declare.

AUTHORS' CONTRIBUTIONS

The cranberry extract's antioxidant and anti-diabetic properties were studied by Mohga A. Ibrahem, Aysam Fayed, and Tamer Roshdy. Soha A. Hassan carried out the histopathological analysis. Mohga A. Ibrahem, Aysam Fayed, Mohammed A. Hussein, and Tamer Roshdy collaborated to write the protocol, the initial draught of the article, manage the study's analysis, and manage the literature searches. The final manuscript was reviewed and approved by all authors.

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