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Biochemistry

Effect of Oral Administration of Cherry and/or Pomegranate Juices on Metabolic and Genetic Consequences in Obese Rats

Nourhan Gamal EL-Rahmany[1], Mai Elsayed[2*] and Fatma Abd EL-Hamid Khalil[3]

[1]Lecturer assistance in Biochemistry and Nutrition Department, Faculty of Women for Arts, Science and Education, Ain Shams University Egypt
[2]Lecturer of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education, Ain Shams University Egypt
[3]Prof. of Nutrition, Biochemistry and Nutrition Department, Faculty of Women for Arts, Science and Education, Ain Shams University Cairo, Egypt

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***Corresponding author:** Mai Elsayed

Abstract **Original Research Article**

The present study was designed to investigate the effect of oral administration of cherry and/or pomegranate juices on obesity associated metabolic disturbances .High fat high sucrose diet (HFHS) was used to induce obesity, experimental groups were; healthy control (G1), obese control (G_2), cherry (G_3), pomegranate (G_4),mix (G_5) and chromium (G_6) groups. The results showed that oral administration of cherry and pomegranate juices significantly reduced body weight and visceral adipose tissue size, also reduced adipocytokines level (interleukin-6, calprotectin, afamin, and visfatin).Obesity caused significant elevation in serum glucose, insulin and leptin levels which is associated with elevated insulin resistance index, while administration of juices reversed these elevations. Moreover, they caused amelioration of antioxidant status by reducing malondialdyde (MDA) level and elevating reduced glutathione (GSH) concentration in liver tissue. Furthermore, our results demonstrated that juices supplementation modulated the gut microbiota composition, decreasing the proportion of firmicutes to bacteroides with significant modulation in fecal short chain fatty acids production as compared to obese control group. In addition, obesity caused a significant increase in steroyl regulatory element binding protein (SREBP) mRNA expression level and reduction in peroxisome proliferator activated receptor (PPAR) mRNA expression level in liver tissue also; it caused a significant increase in serum pancreatic lipase and α-amylase activities with disrupted lipid profile levels. However, juices treatment down regulated hepatic gene expression of SREBP and up-regulated PPAR expression, decreased pancreatic lipase and α-amylase activities along with decreasing serum lipid profile. Meanwhile, administration of juices reduced the visceral fat thickness and mean adipocyte diameter that increased in obese rats. The present results concluded that administration of juices with HFHS diet achieved modulating effects on lipid and glucose metabolism as well as modulating the composition and metabolic activity of intestinal microbiota, ameliorating dyslipidemia and inflammation associated with obesity.

Key words: HFHS diet, SREBP, Microbiota, PPAR, Inflammation, Pomegranate, Cherry juice, Obesity, Leptin.

INTRODUCTION

Obesity is a chronic metabolic disorder that results from an energy imbalance where energy intake is greater than energy expenditure. It is characterized by excessive fat mass and elevated lipid concentration in blood. Several factors have been associated with the increasing prevalence of obesity, including diminished physical exercise and an increased consumption of saturated fats and refined carbohydrates [1]. Obesity is a condition characterized by the excessive accumulation and storage of fat in the body leading to reduced life expectancy and/ or increased health problems **[**2**].**

Oxidative stress is an important pathogenic mechanism of obesity associated metabolic syndrome. Moreover, obesity is associated with disorders in carbohydrate and lipid metabolism. The changes in the number and size of adipocytes affect the microenvironment of expanded fat tissues and are accompanied by alterations in adipokine secretion, adipocyte death, local hypoxia, and fatty acid fluxes [3]**.** Adipose tissue is known to produce and secrete a variety of bioactive substances known as adipocytokines, among which adiponectin and leptin are the predominant, leptin level correlates with adiposity [4]**.**These characteristic metabolic alterations are mediated by the crucial hormonal disturbances in

leptin and insulin, but more prominently by the unrestrained expression of lipid metabolism controlling genes. The expression lipogenic related genes is controlled through direct interaction of energy yielding nutrients and their secondary metabolites with nuclear transcription factors**.**Sterol regulatory element binding protein (SREBP) and peroxisome proliferator activated receptor (PPAR) are important family of transcription factors that are key regulators of lipid metabolism and regulate the expression of genes involved in fatty acids, triacylglycerols and cholesterol metabolism, especially in the liver **[**5, 6].

Obesity is a particularly challenging medical condition because of its complex etiology and adverse health effects. Sustainable agents from natural sources could serve as viable alternatives to currently available synthetic drugs in the management of obesity. Despite short term benefits of synthetic drugs in treatment of obesity, it is often associated with undesired side effects from the medication and rebound weight gain after the cessation of drug use [7].

The identification of substances that are able to decrease or even prevent obesity has become a major goal of research. The increased intakes of fruits and vegetables have been shown to inversely correlate with incidence of obesity. In addition to the benefits from fiber consumption, dietary polyphenolic compounds in fruits and vegetables are promising dietary candidate to control obesity and its associated metabolic complications [8, 9]. Most studies on the treatment of obesity have focused on the potential role of plants and their phytochemical constituents; such as flavonoids and anthocyanins for their anti-inflammatory activity as it can be used for obesity and its metabolic disorders treatments by exerting a positive effect on lipid and glucose metabolism [10, 11]**.** In theory, a combination of a multiple natural products may result in a synergistic activity due to the increased bioavailability and the targeting of multiple molecular pathways, offering advantages over other treatments of obesity [1]**.**

Many dietary supplements, such as polyphenol-rich fruit extracts, can improve the efficacy of interventions targeting the gut microbiota by selectively changing the levels of microbiota and alter the production of a variety of metabolites, such as short-chain fatty acids (SCFAs), by the gut microbiota. Therefore, the gut microbiota represents a potential target for the development of therapeutic drugs or nutritional interventions for obesity [12]**.**

Cherry (*Prunus avium)* is a fruit belonging to the genus *Prunus* in the *Rosaceae* family. Cherries are very rich in polyphenols including: flavonoids (anthocyanins, flavan-3-ols, and flavonols), hydroxycinnamic acids and hydroxybenzoic acids. Among all of these compounds, anthocyanins are responsible for the red color of the fruit and their high bioactivity. Anthocyanin composition in cherries includes, cyanidin 3-glucoside, cyaniding-3-rutinoside, peonidin 3-rutinoside, pelargonidin 3-rutinoside and 3-glucoside [13]**.** Many health benefits have been reported to this fruit, due to its antioxidant activity, anti-proliferative and anti-cancer properties, and anti-inflammatory effects [14, 15].

Pomegranate (*Punica granatum*) belongs to genus *Punica* in the *Punicaceae* family. Pomegranate fruit possess strong anti-inflammatory, antioxidant, anti-microbial, anti-tumoral, neuroprotective properties. These beneficial effects are attributed to the presence of ellagic acid, ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanins, estrogenic flavonols, and flavones. Remarkably, the synergistic action of all these pomegranate constituents appears to be superior when compared to that of the individual constituents [16].

The present study was conducted to investigate the effect of oral administration of cherry and/or pomegranate juices comparing that with chromium on the consequences of obesity including disturbances of lipid and carbohydrate metabolism, oxidative stress and inflammatory reactions.

MATERIALS AND METHODS

Materials

Fruits

The fresh mature cherry (*Prunus avium*) and pomegranate (*Punica granatum*) were purchased from the Ministry of Agriculture, Cairo University (Giza, Egypt), since season August to October 2017. The fruits were carefully selected in term of shape, color and ripeness.

Chemicals

Chromium (Cr) Picolinate drug was obtained from pharmaceutical company, Mepaco, Egypt.

Animals

Sixty adult male rats; Wistar Albino rats, weighing 180 ± 5 g were used in this study. Animals were obtained from Breeding Unit of the Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt).

Diet

Standard diet: Standard diet was prepared to meet the rat's nutrient requirements according to American Institute of Nutrition (AIN- 93 Maintenance diet) and adjusted by Reeves *et al*. [17].

High fat high sucrose diet (HFHS): High fat high sucrose diet was prepared by modifying diet materials for induction of obesity by adding 55% carbohydrates (37% sucrose and 18% starch) and 21%

beef tallow to standard diet according to Yang *et al*. [18] with slight modification.

Methods

Preparation of Cherry and Pomegranate juices

Edible portions of fresh cherry and pomegranate fruits were squeezed using a commercial blender, filtered, then obtained juices are diluted as (1:1; water: pomace) to be given as 1 ml for each rat daily via a gastric tube according to Rouhi *et al.* [19].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Total Phenolic Content in cherry and pomegranate juices were determined and identified by GC-MS. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram, using the Wiley 7 Nist05.L mass spectrometer data base to identify individual compounds according to the method reported by **Falah and Farouk** [20]**.**

Quantitative detection of total polyphenols, flavonoids and anthocyanins in juices

Total phenolic content was determined by using Folin-Ciocalteau method while, Aluminium chloride colorimetric method was used for determination of total flavonoids as described by Ivanova *et al*. [21]. On the other hand,total anthocyanins were calculated according to Garcia-Viguera *et al*. [22].

Preparation of chromium dose

Chromium was administered at a concentration of 110 µg/kg/day dissolved in the drinking water containing 5.53 µg Cr/L for 12 weeks to get 8 µg Cr/day according to Selcuk *et al*. [23].

Experimental Design

Rats were housed individually in animal care facility in constant environment. Animals were allowed seven days period to be adapted to the laboratory conditions. After acclimation, rats were randomly divided into two main sections, group one; 10 rats were provided with standard diet and water ad libitum. While section two; 50 rats received a high fat high sucrose diet (for induction of obesity) and water ad libitum. Induction of obesity was confirmed by calculation of lee index and BMI. After the induction of obesity, rats in section 2 continued to receive HFHS and further divided into five subgroups; 2, 3, 4, 5&6 with 10 rats each. The oral doses were given daily using intragastric intubation (p.o.). The groups were classified as follows (as shown in fig. 1):

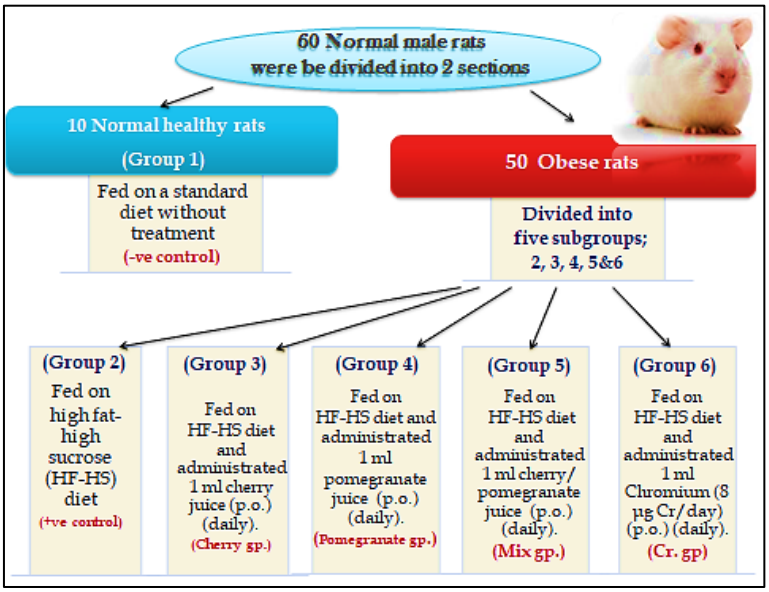

Fig-1: Experimental design.

Samples collection

After six weeks of treatments, rats were fasted for 12 hours, anesthetized and sacrificed under ether anesthesia. Blood samples were taken from hepatic portal vein and serum was separated and part of serum is immediately used for determination of glucose level by colorimetric method. The remaining samples were kept at -20°C until other biochemical analysis. Liver tissue was stored at -20°C for estimation of biochemical parameters. While those for molecular assays were stored at -80 °C until use for gene expression measurement using the realtime PCR technique. White adipose tissue (visceral) was preserved in 10% formalin solution for microscopic examination. Fecal samples were collected for apparent digestibility measurements, GC-mass analysis of SCFA and some are stored at -80 °C for firmicuties and bacteriodes analysis by using PCR technique.

Anthropometric measurements

The body weight of each rat was measured at weekly intervals using electrical balance. Rats' body length (nose—anus length) was measured using non-stretchable tape for calculation of lee index and body mass index (BMI). Lee index is calculated as cubic route body weight (g) divided by the length (cm); where > 0.30 considered obese. The BMI was calculated as body weight (g) divided by the square of the anal-nasal length (cm); where > 0.68 considered obese.

Biochemical analysis

Serum analysis

Serum visfatin, leptin, IL-6 and calprotectin assay were performed following the quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique according to the method described by Leng *et al*. [24], Chessler *et al.* [25],Hirano [26] and Striz and Trebichavsky [27], respectively using CusaBio kits, USA, while using Biovision kits, USA for IL-6 assay. Serum afamin level was measured according to the ELISA technique described by Voegele *et al.* [28] using Cloud-Clone Corp. kits, USA. Serum pancreatic lipase and α-amylase enzyme were performed following ELISA technique according to the method described by Tsuzuki *et al*. [29] and Chessler *et al*. [25], respectively using CusaBio kits, USA.

Serum glucose level was measured by the enzymatic colorimetric method described by Trinder, [30] using Salucea kit, Netherlands. Serum insulin level was measured by quantitative immunoassay technique described by Sacks [31] using CusaBio kits, USA. The homeostasis model assessment of basal insulin resistance (HOMA-IR) was calculated according to the method of Matthews *et al*. [32].

Reduced glutathione (GSH) and malondialdehyde (MDA) concentration were determined in liver homogenate using colorimetric method, kits developed by the Ben Italy Company according the procedure of Beutler *et al*. [33] and Ohkawa *et al*. [34], respectively. Serum total cholesterol, triacylglycerol (TAGs), and high-density lipoprotein cholesterol (HDL-C) were determined following the colorimetric methods of Allain *et al*. [35], Fassati and Prencipe, [36], and Lopez-Virella *et al*. [37], respectively. Serum very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) concentration was calculated according to Friedewald *et al*. [38].While, atherogenic coefficient and atherogenic index risk ratio were calculated as described by Brehm *et al*. [39] and Ridker *et al*. [40] using the following equations:

$$\text{Atherogenic index} = \frac{\text{TC} - \text{HDLc}}{\text{HDL} - \text{C}} \qquad \text{Risk ratio} = \frac{\text{LDL} - \text{C}}{\text{HDL} - \text{C}}$$

Fecal analysis

Apparent digestibility measurements

Apparent digestibility measurements which involved; fecal weight, pH and lipids were determined. Fecal lipids were extracted with chloroform/ methanol mixture (2v/1v) according to the method of Deshpande *et al.* [41].

Gut microbiota distribution

Fecal DNA was extracted and purified from two pellets of fecal sample using phenol-chloroform method by Abbaszadegan *et al*. [42] with practical modification. The extracted DNA was stored at -20˚C for detection of bacteroidetes and firmicutes by PCR technique according to Ismail *et al*. [43]. PCR thermal cycling was done using a Biometra PCR system. The primer sequences are as follows:

Gene	Primers	
Bacteriodes	Forward sequence	5′ ACGCTAGCTACAGGCTTAACA 3′
	Reverse sequence	5′ ACGCTACTTGGCTGGTTCA 3′
Firmicutes	Forward sequence	5′ GCGTGAGTGAAGAAGT 3′
	Reverse sequence	5′ CTACGCTCCCTTTACAC 3′

The intensities of amplified band were determined from the gel electrophoresis photos to estimate integral band density by Gel-Pro Analyzer version 3.1 as described by Heras *et al*. [44].

Fecal short chain fatty acid (SCFA) quantification using Gas Chromatography-mass spectrometer (GC-MS) analysis

For quantitation of SCFA, the metabolic profiling analysis was conducted on a GC-MS system. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram, using the Wiley 7 Nist05.L mass spectrometer data base to identify individual compounds as described by EL-Beltagi and EL-Rahim [45] with modification. Concentrations of acetate, propionate and butyrate were expressed in µmol/g of feces. Total SCFAs were the sum of acetate, propionate, and butyrate.

Analysis of gene expression

Total RNA was extracted from 100 mg of each liver tissue sample using TRIzol total RNA extraction reagent following the methodology of TRIzol kit. The resultant cDNA was preserved at −20 °C until used. The expression of some genes involved in energy metabolism was tested by semi-quantitative polymerase chain reaction (PCR) using specific primers for these genes. The tested genes were sterol regulatory element-binding protein (SREBP) and perixsome proliferator

activated receptor (PPAR). Polymerase chain reaction (PCR) was applied using the Thermo Scientific Dream Taq Green PCR Master Mix (2X) as described by Park *et al*. [46] with a modification of the annealing temperatures, that corresponding to each primer. As a reference (housekeeping gene); expression of β-actin mRNA was detected using specific primers. Relative quantification of expressed genes calculated according to Derveaux *et al*. [47] as follows: $R = 2^{-\Delta\Delta Ct}$. The primer sequences are as follows:

Gene	Primers	
SREBP	Forward sequence	5′ GGAGCCATGGATTGCACATT 3′
	Reverse sequence	5′ AGGAAGGCTTCCAGAGAGGA 3′
PPAR	Forward sequence	5′ TTCGGAATCAGCTCTGTGGA 3′
	Reverse sequence	5′ CCATTGGGTCAGCTCTTGTG 3′
β-actin	Forward sequence	5′ ATGTACGTAGCCATCCAGGC 3′
	Reverse sequence	5′ TCCACACAGAGTACTTGCGC 3′

Microscopic examination of white adipose tissues

Freshly isolated white adipose tissues (WAT, visceral adipose tissue) from all rats were rapidly fixed in 10% formalin and stained with haematoxylin–eosin stain. The stained tissue sections were examined under bright-field microscopy (Olympus Optical, Japan)at 200× magnification. Image-Pro plus Version 5.0 (Media Cyberetics) was used to measure adipocyte average diameters as described by Wu *et al*. [48]. All histological procedures were conducted at the Veterinary Medical Laboratory- Cairo University, Egypt.

Statistical analysis

Statistical analysis of results, were done using analytical software named SPSS statistics 17.0, Chicago, USA. Values were expressed as means ± S.D. Quantitative differences between values were statistically analyzed by one way ANOVA, P values <0.05 were considered to be significant according to **Levesque** [49].

RESULTS

Phytochemical and antioxidant analysis of the pomegranate and cherry juices:

Results of phytochemical and antioxidant analysis showed that the values of total polyphenols measured in mg as gallic acid equivalent; GAE %, total flavonoids in mg as catechin equivalent; CE % and total anthocyanins percentage. Data showed that 1gram of cherry and pomegranate juices contains 47.35mg and 56.43 mg GAE, while contains 41.73 mg and 34.44 mg CE, respectively. In addition, total anthocyanins percentage was 73.65 and 31.14 %, respectively.

Gas chromatography mass spectrometry (GC-MS) was performed for qualitative screening of phenolic compounds in the cherry and pomegranate juices used in the study. Results showed that cherry juice sample contains catechin, epicatechin, rutin, reseveratrol, cyanidine-3 glucoside, apigenin, genistein, quercetin, kaempferol, peonidin, melatonin, caffeic acid and gallic acid. While, results showed that pomegranate juice sample contains punicalagin, pyrogallol, quercetin, catechin, epicatechin, chlorogenic acid, chatechol, P-OH-benzoic acid, ellagic acid, P-coumaric acid, ferulic acid gallic acid, caffeic acid and ellagitanins.

Effect of oral administration of cherry and/or pomegranate juices on the anthropometric measurements of experimental groups:

The results of the present study recorded significant increase ($p \leq 0.05$) in the body weight, lee index, and BMI in obese control group with respect to the healthy control group .Meanwhile, there is significant decrease ($p \leq 0.05$) in body weight, lee index, and BMI in treated groups relative to the obese control group as presented in table (1) with the highest effect was induced by mix juice and chromium administration.

Table-1: Effect of oral administration of cherry and/or pomegranate juices on body weight change, naso-anal length, body mass index and lee index in experimental groups:

Groups	Parameters			
	Body weight change (g)	**Naso-anal length (cm)**	**Final BMI**	**Final Lee index**
Healthy control (-ve control; G1)	18.40 ± 3.6 ^{a}	19.40 ± 1.50 ^{a}	0.64 ± 0.05 ^{a}	0.31 ± 0.01 ^{a}
Obese control(+ve control; G2)	46.90 ± 17.2 ^{b}	19.30 ± 1.56 ^{a}	0.96 ± 0.15 ^{b}	0.36± 0.01 ^{b}
Cherry group(G3)	-50.50 ± 16.7 ^{c}	19.90 ± 1.66 ^{a}	0.65 ± 0.12 ^{a,c}	0.31 ± 0.02 ^{a,c}
Pomegranate group(G4)	-56.00 ± 8.8 ^{d}	19.20 ± 1.61 ^{a}	0.64± 0.10 ^{a}	0.31± 0.02 ^{a,c}
Mix group(G5)	-58.20 ± 11.9 ^{e}	18.90 ± 1.19 ^{a}	0.60 ± 0.07 ^{a,d}	0.31 ± 0.01 ^{a,c}
Chromium group(G6)	-61.70 ± 20.6 ^{f}	18.90 ± 0.87 ^{a}	0.58 ± 0.08^{a,e}	0.30 ± 0.01 ^{d}
LSD	1.25	1.17	0.09	0.01

All values are expressed as mean ± SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

Effect of oral administration of cherry and/or pomegranate juices on fecal measurements in experimental groups:

Apparent digestibility measurements of fecal dry weight, pH and total lipids

The present study demonstrated that fecal measurements are affected by the composition of the diet. Results showed that fecal weight and pH were significantly decreased ($p \leq 0.05$) while fecal lipid was significantly increased ($p \leq 0.05$) in obese control group comparative to the healthy control group. It is noticed that fecal weight and lipids was found to be increased significantly ($p \leq 0.05$) in all treated groups as compared with the positive control group and their respective fecal fat content differ. The fecal weight and fat content was found to be the highest in the pomegranate group, followed by mix group and cherry group then, chromium group. Moreover, pH value of obese control group is slightly lower than that of healthy control group. The fecal pH was significantly ($p \leq 0.05$) lower in the juices treated groups as compared to the positive control group. On the other hand, fecal pH values showed that pomegranate group has lowest pH value; this group has higher fecal fatty acid content which explains the results. While there was no significant change in pH in chromium group as compared to obese control group.

Table-2: Effect of oral administration of cherry and/or pomegranate juices on fecal dry weight, pH and total lipids values in the experimental groups

Groups	Parameters		
	Fecal dry weight (g/day)	Fecal pH (mmHg)	Fecal lipid (g)
Healthy control (-ve control; G1)	1.12± 0.10 [a]	7.46± 0.11 [a]	0.72± 0.01 [a]
Obese control (+ve control; G2)	0.91± 0.01 [b]	7.26± 0.07 [b]	0.85± 0.01 [b]
Cherry group(G3)	1.15± 0.15 [a, c, e]	6.56± 0.15 [c]	0.94± 0.02 [c]
Pomegranate group (G4)	1.27± 0.02 [a, c]	6.09± 0.09 [d]	1.09± 0.10 [d]
Mix group (G5)	1.24± 0.02 [c, a]	6.33± 0.15 [e]	0.96± 0.01 [c,e]
Chromium group (G6)	1.08± 0.10 [d, a, e]	7.28± 0.02 [b]	0.75± 0.03 [a, f]
LSD	0.15	0.19	0.06

All values are expressed as mean ±SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$)

Effect of oral administration of cherry and/or pomegranate juices on fecal intestinal microbiota (Firmicutes and Bacteriodes) abundance and fecal SCFAs in experimental groups:

Results in table (3) and figures (2) showed that the density of firmicutes band was significantly increased ($p \leq 0.05$) in obese group as compared to healthy group, while density of bacteriodes band was significantly decreased ($p \leq 0.05$) in obese group as compared to healthy control group. However, firimicutes band density was significantly decreased ($p \leq 0.05$) in all treated groups as compared with obese control group, while bacteriodes band density was significantly increased ($p \leq 0.05$) in all treated groups comparing to obese control group. It is clear that, obesity caused an increased Firmicutes to Bacteroidetes ratio, while juices treatment modulated this ratio thus modulated the gut microbiota of obese rats. Moreover, cherry administration for obese rats caused a marked improvement in distribution of bacteriodes with a marked decrement in the distribution of firmicutes than other treated groups. In comparing chromium to juices treatment, results showed that chromium administration has little or no effect on Firmicutes to Bacteroidetes ratio.

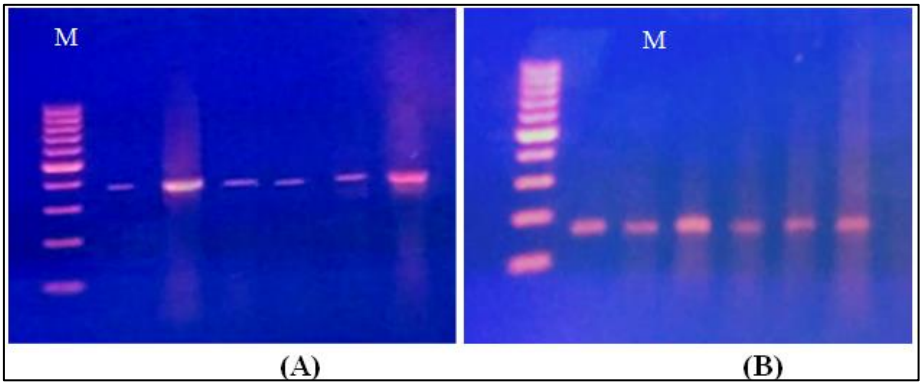

Fig-2: Results of the PCR product bands for Firmicutes (A) and Bacteroidetes (B) by gel documentation system.

Table-3: Effect of oral administration of cherry and/or pomegranate juices on fecal firmicutes and bacteriodes abundance in experimental groups by PCR:

Groups	Parameters	
	Firmicutes	**Bacteriodes**
Healthy control (-ve control; G1)	8.58.± 0.23 [a]	24.87.± 0.4[a]
Obese control(+ve control; G2)	26.38.± 0.19[b]	16.24.± 0.04[b]
Cherry group(G3)	8.68.± 0.04[a]	22.89.± 0.10[c]
Pomegranate group(G4)	10.01.± 0.01[c]	17.41.± 0.13 [d]
Mix group(G5)	9.85.± 0.03 [c]	18.17.± 0.14[e]
Chromium group (G6)	15.21.± 0.03 [d]	16.45.± 0.04[b]
LSD	0.21	0.33

All values are expressed as mean ±SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference (p≤0.05).The intensities of amplified band were determined from the gel electrophoresis photos to estimate integral band density by Gel-Pro Analyzer version 3.1.

Considering, the results presented in table (4), the results of SCFAs level were significantly decreased (p≤0.05) in obese control group as compared to healthy control group. SCFAs content in feces were altered according to diet. Acetic acid was significantly higher in all treated groups as compared to obese control group; there were no significant differences in propionate acid levels among the juices treated groups. Butyric acid was significantly lower in obese control group as compared to all treated groups. The acetic acid, butyric acid, and total SCFA levels in the juices treated groups were found to be significantly increased relative to the obese control group levels, while the levels of these fatty acids in the chromium group did not differ significantly from those in the obese control group. Also, it's clear that, changes in the gut microbiota modulate SCFA production which may be responsible for the lowest fecal pH.

Table-4: Effect of oral administration of cherry and/or pomegranate juices on fecal Short chain fatty acids concentration in the experimental groups:

Groups	Parameters			
	Total SCFA (µmol/g)	**Acetate (µmol/g)**	**Propionate (µmol/g)**	**Butryate (µmol/g)**
Healthy control(-ve control; G1)	2.57 ± 0.25 [a]	2.36± 0.11 [a]	3.97± 0.65 [a]	1.56 ± 0.58 [a]
Obese control(+ve control; G2)	0.16 ± 0.08 [b]	0.39± 0.25 [b]	0.03± 0.02 [b]	0.08± 0.01 [b]
Cherry group (G3)	3.22 ± 0.05 [c]	2.02± 0.06 [c]	3.44± 0.41 [c]	4.20± 0.26 [c]
Pomegranate group (G4)	2.68 ± 0.24 [d, a]	1.50 ± 0.10 [d]	3.46± 0.92 [c]	3.10± 0.20 [c]
Mix group (G5)	3.10 ± 0.48 [e, d]	1.92 ± 0.04 [e]	3.51± 1.25 [c, a]	3.68 ± 0.25 [c]
Chromium group (G6)	0.83± 0.14 [f]	0.80 ± 0.05 [f]	0.59± 0.53 [d]	1.10 ± 0.08 [a.b]
LSD	0.43	0.16	0.48	1.24

All values are expressed as mean ±SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference (p≤0.05).

Effect of oral administration of cherry and/or pomegranate juices on serum interleukin-6, calprotectin, afamin, visfatin and leptin levels in the experimental groups:

Results presented in table (5) showed a significant elevation (p≤0.05) in interleukin-6, calprotectin as well as afamin serum levels of obese control group comparing with the healthy control group. Whereas, treatment with juices significantly reduced (p≤0.05) this elevation as compared to positive control group. The Mix group recorded the most significant decrease than cherry and pomegranate. With respect to serum visfatin and leptin level, obese control group showed a significant increase (p≤0.05) in serum visfatin and leptin level as compared to healthy control group. However, in comparing the treated groups with the obese control group a significant decrease (p≤0.05) was observed in all treated groups. The most significant decrease was shown in Mix group thanG3 and G4.

Table-5: Effect of oral administration of cherry and/or pomegranate juices on serum interleukin-6, calprotectin, afamin, visfatin and leptin levels in the experimental groups:

Groups	Parameters				
	Interleukin-6 (pg/ ml)	**Calprotectin** (ng/ ml)	**Afamin** (ng/ ml)	**Visfatin** (ng/ ml)	**Leptin** (ng/ ml)
Healthy control (-ve control; G1)	130.26± 5.30 [a]	278.47± 7.76 [a]	37.97± 1.47 [a]	9.89± 0.85 [a]	11.43± 0.56 [a]
Obese control (+ve control; G2)	233.46± 5.38 [b]	604.53± 4.07 [b]	69.66± 2.58 [b]	35.03± 1.07 [b]	19.22± 0.65 [b]
Cherry group (G3)	194.80± 2.34 [c]	511.31± 4.34 [c]	61.79± 0.64 [c]	23.37± 0.40 [c]	18.03± 0.06 [c]
Pomegranate group (G4)	201.91± 3.39 [d]	459.22± 3.75 [d]	58.54± 0.88 [d]	25.65± 0.42 [d]	17.29± 0.38 [d]
Mix group (G5)	175.51± 3.04 [e]	401.35± 4.08 [e]	54.35± 1.31 [e]	17.60± 0.63 [e]	16.99± 0.32 [e,d]
Chromium group (G6)	209.12± 2.47 [f]	566.00± 5.03 [f]	61.90± 1.04 [c,f]	28.97± 0.87 [f]	15.87± 0.24 [f]
LSD	3.38	4.40	1.28	0.65	0.36

All values are expressed as mean ±SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference (p≤0.05).

Effect of oral administration of cherry and/or pomegranate juices on serum pancreatic lipase and α-amylase enzyme activities in the experimental groups

From the current results; table (6) showed that, obesity caused a significant increase (p≤0.05) in pancreatic lipase and α-amylase activities as compared to healthy control group. Compared to obese control group, serum activities of pancreatic lipase and α-amylase activities were significantly decreased (p≤0.05) in treated groups. While, the most significant decrease was observed in pomegranate administered group followed by mix administered group. Results showed that juices effect was better than chromium effect. The decreased lipase activity in all treated groups is in line with increased fecal fat excretion as revealed before.

Table-6: Effect of oral administration of cherry and/or pomegranate juiceson serum pancreatic enzymes (lipase and α-amylase) activities in the experimental groups:

Groups	Parameters	
	Pancreatic lipase (U/ml)	**Pancreaticα- amylase (mIU/ ml)**
Healthy control (-ve control; G1)	16.45± 0.89 [a]	19.47± 1.74 [a]
Obese control(+ve control; G2)	32.43± 1.12 [b]	43.27± 1.30 [b]
Cherry group(G3)	30.35± 0.69 [c]	39.07± 1.20 [c]
Pomegranate group(G4)	25.35± 1.85 [d]	26.19± 1.02 [d]
Mix group(G5)	27.09± 0.53 [e]	33.99± 0.30 [e]
Chromium group (G6)	28.76± 0.37 [f]	36.39± 0.92 [f]
LSD	0.90	1.02

All values are expressed as mean ±SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference (p≤0.05).

Effect of oral administration of cherry and/or pomegranate juices on serum glucose homeostasis of experimental groups

Insulin resistance in the liver is a frequent metabolic alteration associated with defective insulin signaling observed during diet-induced obesity. In the obese control group, there were a significant increase (p≤0.05) in serum glucose level, serum insulin and insulin resistance value compared to the healthy control group as shown in table (7). Obesity caused a disruption in glucose homeostasis while treatment reversed these changes. With respect to insulin level, results showed a significant reduction (p≤0.05) in the insulin level in all treated groups as compared to obese control group and this is in line with the results of HOMA-IR, that showed significant reduction (p≤0.05) in the mean values of HOMA-IR observed in all treated groups (G3, G4, G5 and G6) as compared to obese control rats. However, comparing the juices treated group with the chromium group, results showed that the effect of chromium on treatment of insulin resistance was more powerful than juices. While, Mix group followed by pomegranate group showed a significant decrease (p≤0.05) in serum glucose, serum insulin and HOMA-IR, values as compared to cherry group. Therefore it is concluded that, juices as well as chromium administration decreased insulin resistance and enhanced glucose utilization and insulin sensitivity in obese rats.

Table-7: Effect of oral administration of cherry and/or pomegranate juices on serum glucose, insulin levels and homeostatic model assessment of insulin resistance in the experimental groups:

Groups	Parameters		
	Glucose level* (mmole/L)	Insulin (U/ml)	HOMA-IR
Healthy control(-ve control; G1)	5.86± 0.31 [a]	24.13± 0.25 [a]	6.27± 0.31 [a]
Obese control(+ve control; G2)	6.78± 0.14 [b]	47.82± 3.66 [b]	14.44± 1.30 [b]
Cherry group(G3)	5.76± 0.35 [a,c]	38.42± 0.71 [c]	9.51± 0.37 [c]
Pomegranate group (G4)	5.57± 0.20 [a,c]	34.8± 0.23 [d]	8.92± 0.55 [d]
Mix group(G5)	5.37± 0.21 [a,c]	31.76± 0.13 [e]	7.58± 0.30 [e]
Chromium group(G6)	5.26± 0.39 [a,c]	31.25± 0.43 [f,e]	7.31± 0.54 [f,e]
LSD	0.24	1.34	0.57

All values are expressed as mean ±SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).Glucose values are converted to mmol/l for calculating HOMA-IR, where (1 mmol/l=18 mg/dl)*

Effect of oral administration of cherry and/or pomegranate juices on serum lipid profile analysis in the experimental groups

It is clear from this current data that, obesity significantly affected lipid profile as observed by the results of this study and shown in table (8), the increase in LDL-c, atherogenic coefficient and atherogenic risk, was inhibited by juices administration and HDL-c level was significantly increased. On the other hand, the elevation in serum levels of TAG, cholesterol and VLDL observed in obese group was significantly decreased; results showed best hypolipidemic effect was seen in chromium and mix group.

Table -8: Effect of oral administration of cherry and/or pomegranate juices on serum lipid profile levels, atherogenic coefficient and atherogenic risk in the experimental groups

Groups	Parameters						
	TAG (mg/dl)	TC (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	Atherogenic coefficient	Atherogenic risk
Healthy control (-ve control; G1)	61.55± 2.49 [a]	167.85± 3.54 [a]	50.38± 2.90 [a]	106.24± 6.45 [a]	12.31± 0.49 [a]	2.34± 0.23 [a]	2.11± 0.21 [a]
Obese control (+ve control; G2)	124.82± 2.25 [b]	219.65± 5.13 [b]	36.96± 2.52 [b]	159.42± 4.75 [b]	24.96± 0.45 [b]	4.96 ± 0.35 [b]	4.33± 0.39 [b]
Cherry group (G3)	80.69± 3.00 [c]	179.27± 5.24 [c]	54.45± 2.27 [d]	120.12± 4.81 [c]	17.73± 0.60 [c]	3.61 ± 0.33 [a,b]	3.14± 0.29 [a]
Pomegranate group (G4)	78.97± 4.26 [d]	177.79± 3.93 [c]	48.20± 3.94 [a,c]	118.83± 6.60 [c]	15.79± 0.85 [d]	2.67± 0.27 [a]	2.35± 0.25 [a]
Mix group (G5)	77.12± 4.77 [d,e]	174.62± 5.02 [d,c]	47.72± 3.12 [e]	118.76± 5.34 [c]	15.42± 0.95 [e]	2.64± 0.29[a]	2.31± 0.26 [a]
Chromium group (G6)	70.89± 2.91 [f]	170.53± 5.75 [e,d,c]	39.01± 2.80 [b]	112.32± 4.71 [d]	13.98± 0.58 [f]	2.26 ± 0.15 [a]	1.97± 0.13 [a]
LSD	2.98	4.23	2.60	4.81	0.59	1.82	1.74

All values are expressed as mean ±SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

Effect of oral administration of cherry and/or pomegranate juices on oxidative stress status in liver tissue of experimental groups

Oxidative stress markers measured in liver tissue homogenates that illustrated in table (9) showed that GSH content was significantly decreased ($p \leq 0.05$) with significant elevation in MDA level in obese control rats as compared to healthy control rats. Remarkably, cherry, pomegranate, mix and chromium treatments caused a significant increase in GSH content as compared to positive control group. In the present study all administrated juices is accompanied with elevation in the GSH concentration and decrease in the MDA concentration caused by obesity condition compared to chromium group. It's concluded that best amelioration to oxidative stress caused by obesity was observed in cherry treated group which caused a significant elevation of GSH content while, caused a significant reduction in MDA content.

Table-9: Effect of oral administration of cherry and/or pomegranate juices on hepatic malondialdehyde and reduced glutathione levels in the experimental groups

Groups	Parameters	
	MDA (nmol/g tissue)	GSH (mg/g tissue)
Healthy control(-ve control; G1)	3.46± 0.25 [a]	95.94± 1.65 [a]
Obese control(+ve control; G2)	15.56± 1.12 [b]	64.55± 2.27 [b]
Cherry group(G3)	4.88± 0.32 [c]	90.34± 3.18 [c]
Pomegranate group(G4)	5.15± 0.19 [c]	82.97± 2.81 [d]
Mix group(G5)	4.97± 0.25 [c]	83.38± 2.02 [d]
Chromium group(G6)	7.08± 0.49 [d]	77.15± 3.76 [e]
LSD	0.47	2.38

All values are expressed as mean ±SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

Effect of oral administration of cherry and/or pomegranate juices on the gene expression of Steroyl regulatory element binding protein (SREBP) and Peroxisome proliferator activated receptor (PPAR) in liver of experimental groups

Results in table (10) showed that obesity caused a significant increase ($p \leq 0.05$) in SREBP transcriptional activity, inducing lipid synthesis and triacylglycerols accumulation in the liver compared to healthy control group. Whereas, juices and chromium administration significantly reduced ($p \leq 0.05$) mRNA expression of SREBP as compared to obese control group. Furthermore, PPAR is a transcription factor that is involved in the control of the gene network and regulates glucose-stimulated insulin secretion in pancreatic β-cells and regulate fatty acid oxidation. In positive control group the PPAR mRNA expression is significantly down regulated ($p \leq 0.05$) thus, reduced the mitochondrial fatty acid oxidation as compared to healthy control group. In treated groups (G3, G4, G5 and G6), PPAR mRNA expression is significantly up-regulated ($p \leq 0.05$) inducing fatty acid oxidation and modulate insulin secretion as compared to obese control group. Concerning the results, the mix and chromium group was the most significant in down-regulation of SREBP and up-regulation of PPAR thereby affecting lipogenesis and lipolysis modulating the disturbance in hepatic lipid and carbohydrate metabolism caused by obesity and act in preventing lipid accumulation.

Table-10: Effect of oral administration of cherry and/or pomegranate juices on steroyl regulatory element binding protein (SREBP) and peroxisome proliferator activated receptor (PPAR) gene expression in liver of the experimental groups:

Groups	Parameter	
	Relative gene expression of (SREBP/Hβ-actin)	Relative gene expression of (PPAR/Hβ-actin)
Healthy control(-ve control; G1)	1.00± 0 [a]	1.00± 0 [a]
Obese control(+ve control; G2)	2.56± 0.82 [b]	0.54± 0.33 [b]
Cherry group(G3)	1.37± 0.02 [a]	1.28± 0.21 [a]
Pomegranate group (G4)	1.33± 0.08 [a]	1.27± 0.30 [a]
Mix group(G5)	1.31± 0.02 [a]	1.38± 0.11 [a,c]
Chromium group(G6)	1.21±0.02 [a]	1.50± 0.30 [c]
LSD	0.58	0.39

All values are expressed as mean ±SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

Microscopic findings

The obtained figures showed that, HFHS diet induces hypertrophy of the adipocytes, while the adipocytes cell size was markedly decreased when rats are treated with different juices and chromium. Normal adipocytes distribution with regular sizes of cells of the rats was shown in the healthy control group (G1) fig. (3).While, the large expansion of adipocytes was measured in the positive control group (G2) fig. (4). On the other hand, the size of adipocytes as presented in table (11) is significantly increased ($p \leq 0.05$) in positive control (G2) as compared to healthy control, while G3, G4, G5 and G6 treated rats showed significant decrease ($p \leq 0.05$) in visceral fat thickness and mean adipocyte diameter as compared to positive control group (G2).Protective effect of juices and chromium against expansion of adipose tissue by obesity was shown in figures (5, 6, 7 and 8). Pomegranate group showed better preservation of adipose tissue structure and size than other juices as well as chromium group. The results of biochemical analysis were in line with microscopic observations of WAT sections.

Table-11: Effect of oral administration of cherry and/or pomegranate juices on adipocyte size in the experimental groups

Groups	Parameters
	Adipocyte size (mm)
Healthy control(-ve control; G1)	34.83± 5.06 [a]
Obese control(+ve control; G2)	134.16± 5.14 [b]
Cherry group(G3)	47.16± 3.03 [c]
Pomegranate group (G4)	43.16± 2.47[d]
Mix group(G5)	46.20± 3.63 [e,c]
Chromium group(G6)	36.88± 3.17 [f]
LSD	3.40

All values are expressed as mean ±SD, n=10, there was no significant difference between means have the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

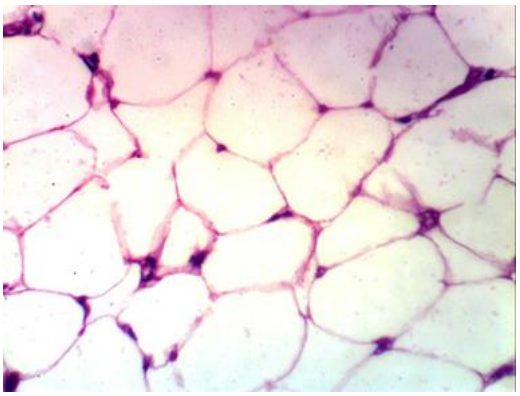

Fig-3: Normal control group

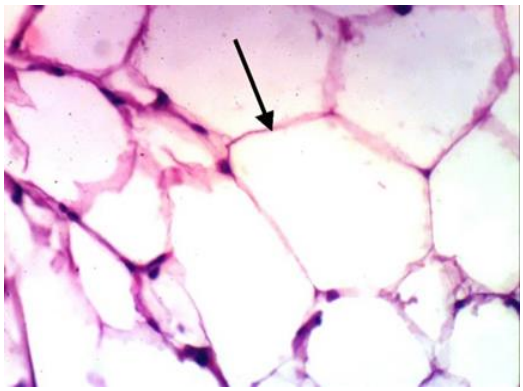

Fig-4: Obese control

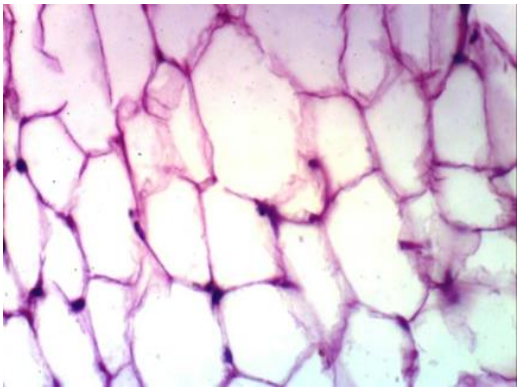

Fig-5: Cherry group

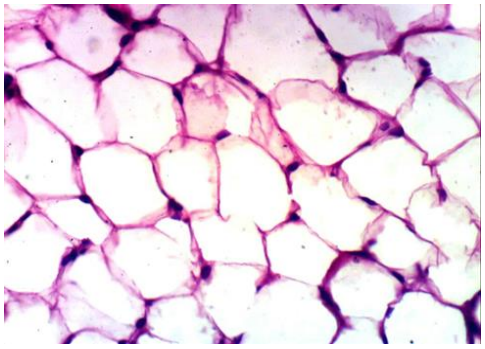

Fig-6: Pomegranate group

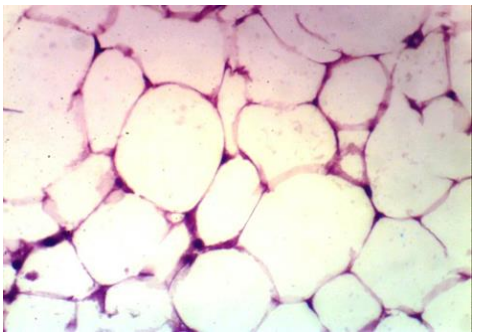

Fig-7: Mix group

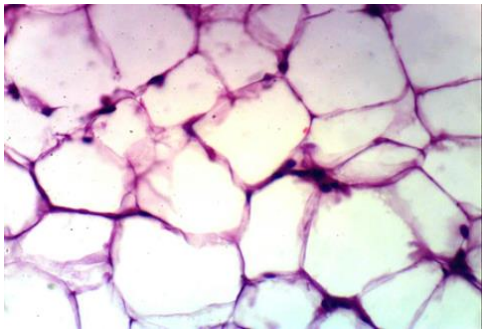

Fig-8: Chromium group

DISCUSSION

Plant-based foods and vegetarian diet are high in many dietary phytochemicals which appear to be related to the lower body weight and risk of metabolic disorders. Phytochemical-rich foods have lower calorie and glycemic index which explain the reduced risk of obesity. Moreover, plant phytochemical has strong anti-obesity effects by targeting adipocyte lifecycle in different ways [50]. In the present study, consuming pomegranate and cherry juices effectively reduced adiposity and its metabolic dysfunctions in obese rats by modulating signaling pathways that regulate lipid metabolism, enhancing anti-inflammatory activities as well as modulating gut microbiota diversity and metabolites. The Phytochemical analysis of cherry and pomegranate juices showed various bioactive compounds, especially phenolic compounds reflecting the antioxidant capacity of these fruits.

It has been reported that multiple mechanisms are involved in decreasing body weight,the beneficial

effects associated with the consumption of polyphenols have demonstrated the anti-oxidant and anti-inflammatory activities of polyphenolics, some of which have also been shown to possess anti-lipidemic and anti-obesity effects, including suppression of adipogenesis and adipocyte proliferation, inhibition of fat absorption, as well as modulation of energy metabolism and inflammation. A flavonoid sub-class with potential for anti-obesity-related effects is the anthocyanins, responsible for the red, blue and purple colors in fruits and vegetables [51].

Flavonoids can modulate various metabolic pathways in the management of obesity including: energy intake and expenditure, lipid absorption by inhibiting pancreatic lipase, increasing lipolysis, and decreasing lipogenesis as well as differentiation and proliferation of pre-adipocytes [11]**.** Furthermore, anthocyanins and epicatechins play a role in modulating the composition and metabolic activity of intestinal microorganisms, attenuating dyslipidemia and obesity associated inflammation [52].

Anthocyanins are dietary bioactives that have been shown to reduce inflammation and insulin resistance in obese animals. Cherry and pomegranate juices rich in anthocyanins which is powerful antioxidant, antiinflamatory and lipid lowering effects. In this study, rats fed HFHS diet with cherry and pomegranate juices were shown to have an attenuation of insulin resistance and systemic inflammation compared with obese controls. The effects of non-anthocyanin molecules and the possible synergistic action between anthocyanins and the complex mixture of phytochemicals present in cherry and pomegranate fruits (quercetin, gallic acid, catechin, epicatechin, rutin, reseveratrol, genistein, punicalagin, catechin and epicatechin) as revealed by the results of GC is responsible for pharmacological activity of cherry and pomegranate fruit juices.

Adipose tissue hypertrophy, lipid spillover and hyperleptinemia are thought to be the earliest signs of metabolic alterations during obesity, leading to hepatic and skeletal muscle lipid accumulation and lipotoxicity. Adipocyte size presented a significant correlation with serum leptin content in the all groups. Thus, the current data suggested that the all juices and specifically pomegranate juice is more effective in preventing adipose tissue hypertrophy. The reduced serum leptin level found in the juices-treated obese groups compared to the obese control group can be attributed to reduced visceral adiposity after juices treatment. Leptin is an adipocytokine secreted by adipocyte and its level is well known to proportionate with body fat mass. Moreover, it is possible that juices decreased the body weight through improving leptin and visfatin sensitivity in the treated groups, which resulted in increased energy expenditure and decreased food consumption and consequently reduced body weight.

Inflammation and oxidative stress are the major causal factors implicated in worsening of obesity and its associated complications. Overfeeding or the consumption of a HFHS diet is usually involved in increasing chronic and low-grade inflammation. The inflammatory response initiator in obesity, visceral adipose tissue, could produce and secrete many proteins, which participate in obesity-related derangements [3, 53]. The secretion of proinflammatory cytokines, such as interleukin-6, calprotectin and afamin into circulation can be promoted by triacylglycerols deposition in adipose tissue; in turn, amplifying inflammatory signal contributes to further adiposity and insulin resistance. Calprotectin and afamin are key parameters of the metabolic syndrome, their levels is elevated in obesity.

Resolving inflammation by ameliorating proinflammatory cytokines could improve obesity state. Accumulating evidence indicates that obesity is associated with systemic oxidative stress and low-grade systemic inflammation, whereas consuming polyphenols attenuates these disease conditions. Following consumption of anthocyanins, serum antioxidant status is significantly increased after 4 to 24 hr post-consumption [54]. Results showed that both cherry and pomegranate juices exhibit a synergistic effect on alleviating oxidative stress, inflammation and metabolic distrubance associated with obesity.Results of this study showed that co-administration of juices with HFHS diet significantly reduced serum levels of interleukin-6, calprotectin and afamin. It is concluded that, juices administration is accompanied with modulation of oxidative stress markers measured in liver tissue reflecting the antioxidant and anti-inflammatory effect of juices.

It has been speculated that the protecting effects of phenolics and anthocyanins on LDL oxidation may be due to multiple factors such as scavenging of various radical species in the aqueous phase, interaction with peroxy radicals at the LDL surface and terminating chain-reactions of lipid peroxidation by scavenging lipid radicals and regenerating endogenous α-tocopherol back to its active antioxidative form [54].

Ahmed *et al*. [55] reported that increased lipolytic activity in fat accumulation leads to increase free fatty acids flux to the liver which causing stimulation of gluconeogenesis as well as depletion of insulin effect on peripheral glucose. Furthermore, it has been recorded that obesity is low-grade chronic systemic inflammation which may be led to insulin resistance. Insulin signaling in adipose tissue plays a key role in the storage of lipid as well as glucose homeostasis regulation. Furthermore, adipocytes insulin signaling is critical for obesity development and its associated metabolic abnormalities, and abrogation of insulin signaling in fat unmasks heterogeneity in

adipocyte response in terms of gene expression as well as the storage of TAGs.

The increased body weight in obese control group was accompanied by changes in serum lipid profile as observed by the results of this study. The body weight lowering effect of juices was accompanied by decrease in the food consumption of the treated groups proving the more significant hypolipidemic effect of juices, which are similar to results obtained by Ahmed *et al*. [7].

Rouhi *et al*. [19] reported that ellagic acid, catechins and other compounds in the pomegranate juice are responsible for its potent antioxidant activity, anti-inflammatory and lipid lowering effect. Pomegranate juice reduced levels of Il-6, TAG, LDL-c, glucose, enhanced insulin level as well as increased HDL-c level which is in agreement with results of our study. Results of Wu *et al*. [48] concluded that cherry anthocyanins reduced the size of adipocytes, decreased leptin secretion, serum glucose, triacylglycerols, total cholesterol, LDL-c and liver triacylglycerols as well as reduced the expression levels of IL-6 genes.

In addition, juices administration showed beneficial effects on insulin sensitivity, glucose tolerance and insulin resistance, which have been reported to be related to decreased serum level of visfatin, increased PPAR and decreased SREBP expression in liver, along with reduced proinflammatory cytokines concentrations and decreased serum level of TAG in obese rats supplemented with cherry and pomegranate juice. Polyphenols such as resveratrol and catechin reduces body weight gain, increases insulin secretion and increases energy expenditure together with decrease in hepatic and adipose tissue fat accumulation preventing hepatic steatosis and adipocyte hypertrophy [56]. While, Ahmed *et al*. [7] results reported a reduced body weight gain, food consumption, and serum levels of lipid, leptin, glucose, and hepatic mRNA expression of SREBP on pomegranate juice administration thereby, protecting against the high fat diet induced obesity in rats.

Pancreatic lipase is involved in the TAG absorption from the small intestine to the enterocytes and if somehow this initial movement of TAG from the small intestinal lumen is blocked, hyperlipidemia can be prevented. Thus, an inhibitor of digestive lipase that helps to limit intestinal fat absorption could be proved as useful medication for the treatment of hyperlipidemia and holds great promise as an anti- obesity agent [57]**.** Cherry and pomegranate juice decreases serum lipase activity due to its bioactive compounds that reported to inhibit pancreatic lipase such as polyphenols, tannins, proanthocyanidin, and flavonoids contents. The decreased lipase activity in all treated groups is in line with increased fecal fat excretion and enhancing the disturbance occurred in lipid profile by HFHS diet.

Hyperlipidemia can be prevented by an inhibitor of pancreatic lipase that helps to limit intestinal fat absorption could be proved as useful medication for the treatment of hyperlipidemia and regarded as antiobesity agent. Dludla *et al*. [3] investigated the beneficial effects of fruits-rich in gallic acid as pomegranate on ameliorating obesity associated complications by modulation of glucose and lipid metabolism through inhibiting pancreatic lipase and α-amylase. Wu and Tian [58] demonstrated that effect of pomegranate extracts, using in vitro screening tools to suppress α-glucosidase, α-amylase, and lipase activities which are responsible for hypolipidemic and antiobesity effect of pomegranate.

Accumulating evidence has revealed that the gut microbiota is associated with obesity and has been regarded as a potential target for the treatment of chronic diseases. Thus, many dietary supplements have been used to prevent or alleviate metabolic diseases by modulating the gut microbiota. From results of this study, it is concluded that cherry could restore the gut microbiota composition that was destructed by the consumption of a HFHS diet. Results demonstrated that juices supplementation modulated the gut microbiota composition, decreasing firmicutes with modulating the proportion of firmicutes to bacteroides. Whereas, some SCFAs, the end products of gut microbial fermentation, contribute greatly to improving glucose homeostasis, lipid metabolism, appetite, and the immune system. Juices supplementation significantly increased the concentration of total SCFAs, particularly acetic acid and butyric acid in juices supplemented rats.

Also Garcia-Mazcorro *et al*. [59] reported that cherry supplementation can modify gut microbiota diversity and the concentrations of fecal SCFAs thus posses antiobesity effect.Whereas, resveratrol and quercetin polyphenols exhibits anti-inflammatory and anti-obesity effects indicated by results of Zhao *et al*. [60] that consumption of fruits rich in them could reduce the body weight gain, visceral adipose tissue weight, serum lipids and inflammation associated with obesity, it also reduced the abundance of firmicutes.

Moreover, the antiobesity effect of chromium is due to its effect on insulin and molecular pathways affecting adipogenesis. Chromium drug modulates insulin action and glucose homeostasis thereby it is used treats diabetes and obesity disease [61]**.** The possible mechanism of action is that chromium may enhance insulin receptor binding, increase the number of insulin receptors, and insulin receptor phosphorylation, resulting in the reduction of insulin resistance in peripheral tissues. Also, chromium activated DNA methylation and histone modification of zinc finger protein which is an important transcription

factor that activates adipogenic signaling. Over expression of zinc finger protein activated PPAR-ɣ expression in adipose tissue and mitigates adipogenic differentiation, thereby inhibiting lipogenesis and lipid accumulation in adipose cells [62]**.** Chromium supplementation has been also shown to inhibit increase in inflammatory markers and oxidative stress levels that occur due to high glucose levels by activation of Nrf2 which is the main regulator of antioxidant genes [23].

CONCLUSION

In conclusion, results suggest that consuming pomegranate and cherry juices effectively reduced metabolic dysfunctions and adiposity associated with high fat high sucrose diet by modulating signaling pathways that regulate lipid and carbohydrate metabolism, enhancing anti-inflammatory activities as well as modulating gut microbiota diversity and metabolites, and affecting gene response. The possible synergistic action between anthocyanins and the complex mixture of phytochemicals present in cherry and pomegranate fruits attenuated the body weight gain and alleviated oxidative stress, inflammation and metabolic disturbance associated with obesity. This effect is due to various phenolic compounds and anthocyanins present in juices reflecting the antioxidant capacity of these fruits.

Ethical approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All animal experiments were performed under protocol approved by the Local Institutional Animal Ethics Committee of Ain Shams University.

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