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EFFECT OF ANTHOCYANIN RICH VEGETABLES ON HEART DISEASE RISK MARKERS

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ABSTRACT

Heart disease is number one cause of death in the world, and preventing it is at the top of the public health agenda. Anthocyanin-rich foods and beverages and purified anthocyanins improve lipid profiles and several cardiovascular disease (CVD) risk markers, possibly through their anti-inflammatory and anti-oxidative properties. The aim of this study was to extract anthocyanins from red cabbage leaves, red radish root peels and eggplant peels, to find out the optimal levels of anthocyanin rich extracts for coloring yogurt samples. Also, to study the effect of the optimal levels of anthocyanin- rich extract additions on some heart disease risk markers in obese aged rats. It was found that anthocyanin contents in red cabbage leaves, red radish root peels and eggplant peels were 1.43 mg, 0.37 mg and 1.60 mg anthocyanins / g fresh weight, respectively. Addition of 3.6 mg of anthocyanin in 3.8 ml eggplant anthocyanin- rich extract followed by 3.4 mg anthocyanin in 4 ml red cabbage anthocyanin- rich extract and 0.9 mg anthocyanin in 4 ml red radish anthocyanin rich extract resulted in the best coloring for yogurt samples according to sensory evaluation.

Anthocyanin rich extracts caused significant reduction in body weight of obese aged rats. Blood glucose and serum insulin levels significantly decreased after administration of the anthocyanin- rich extracts in obese aged rats compared with obese aged control rats. Administration of anthocyanin- rich extracts caused significant reduction in malondialdehyde level and significant increase in GPx activity. Fibrinogen levels in the obese aged rats fed on the anthocyanin- rich extracts were significantly decreased compared to those of obese aged control rats and thrombin concentrations had the same trend. Administration of anthocyanin -rich extracts resulted in a significant improvement in triglycerides levels and lipid profiles at the end of the experiment.

From the present results, it could be concluded that anthocyanin -rich extracts from different parts of plant materials could be used as alternatives to the use of synthetic dyes for coloring food products with potential health benefits especially for treating and enhancing risk markers of heart diseases and other age-related diseases.

Keywords: *anthocyanin rich extracts, eggplant peels, heart diseases, oxidative stress, red cabbage leaves, red radish root peels, risk markers.*

1. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death and disability in developed nations and is increasing rapidly in the developing world. An estimated 17.1 million people died from cardiovascular disease each year, mainly from heart disease (7.2 million) and stroke (5.7 million). By the year 2030, this number is expected to increase to 23.6 million people and it is estimated that CVD will surpass infectious diseases as the world's leading cause of death and disability (WHO, 2008).

Cardiovascular disease is caused by disorders Of the heart and blood vessels. Many traditional cardiovascular risk factors are associated with the onset of endothelial dysfunction including: hypertension, hypercholesterolemia, smoking, increased age, type 2 diabetes, obesity, insulin resistance and oxidative stress, in addition to nontraditional risk factors, such as inflammatory processes and abnormalities of the blood coagulation system. There is evidence that even modest elevations of blood pressure, cholesterol and glucose levels combine to place individuals at risk for CVD (Sitia *et al.*, 2010).

Consumption of fruit and vegetables has been inversely associated with a decreased risk of CVD. Anthocyanins are red, blue and purple water soluble pigments found in fruits and vegetables. Mean distribution of the six most common anthocyanidins in the edible parts of plants as follows: cyanidin (50%), pelargonidin (12%), delphinidin (12%), peonidin (12%), petunidin (7%) and malvidin (7%). The three non-methylated anthocyanidins: cyanidin, delphinidin and pelargonidin are the most widespread in nature. Recently, a 16 year follow-up study of CVD free postmenopausal women showed that dietary intakes of certain classes of flavonoids, including flavanones and anthocyanins were associated with a reduced risk of death due to heart disease (Clifford, 2000 and Mink *et al.,* 2007).

Color is an important factor for the acceptability of food products. Anthocyanins have the potential to act as natural red, blue and purple colorants that can be used in foods and beverages as alternatives to the use of synthetic dyes, but their use is limited by their poor stability. Acylated anthocyanins, such as those found in purple carrot, red cabbage, red radish, purple sweet potato and purple eggplant have a greater stability than nonacylated anthocyanins and therefore may be used as a source of natural colorants and may be included in challenging systems such as dairy products (Turker *et al.,* 2004).

Red cabbage (*Brassica oleracea* L. var. *Capitata*) is a native crop in the Mediterranean region and now grows all over the world as a fresh market vegetable. The color of red cabbage is due to anthocyanin accumulation which exists in high levels. The amount of total anthocyanins in red cabbage was found to be positively correlated with the total antioxidant power. The predominant anthocyanins are considered as cyanidin-3-diglucoside-5-glucoside "cores" which are nonacylated, monoacylated or diacylated with p-coumaric, caffeic, ferulic and sinapic acids. The degree of thermal stability was red cabbage > black currant > grape skin > elderberry. There were significant losses of anthocyanins in blanched red cabbage, while boiling gave less extensive reduction. In general losses are detected in the processing waters. Long–term freezer storage did not affect the anthocyanins content and only minor effects were found for the color parameters (Arapitsas *et al.,* 2008 and Volden *et al.,* 2009).

Red radish (*Raphanus sativus* L.) is cultivated in southeast Asia. Six new acylated anthocyanins were isolated along with the ten known acylated anthocyanins from the fresh root of red radish. The acylated anthocyanins are all based on pelargonidin-3-sophoroside-5-glucoside, acylated with caffeoyl, feruloyl or p-coumaroyl moieties. The radical scavenging activity of acylated anthocyanins from red radish mostly depended on the activity of intramolecular acyl units, *i.e.*, caffeic acid > ferulic acid > p-coumaric acid (Tamura *et al.,* 2010).

Eggplant (*Solanum melongena* L.) is a plant native in India, and the most widely cultivated varieties are elongated ovoid or slender type in a dark purple skin. Its fruit, commonly known as aubergine, is widely used as a vegetable in cooking. The skin of eggplant contains different kinds of anthocyanins and the major anthocyanin in eggplant is delphinidin-3-rutinoside. Application of two *in vitro* assays for antioxidant capacity assessment revealed that eggplant generally exhibited higher values compared to violet pepper. Eggplant peel showed higher amounts of anthocyanin than pulp and relatively higher reducing power. The inhibitory effect of delphinidin on H2O2-induced lipid peroxidation is about 2-fold stronger than nasunin, the major component of the anthocyanin pigment of eggplant, and has antioxidant activity (Sadilova *et al.,* 2006 and Jung *et al.,* 2011).

The increased stability of anthocyanin sources rich in cyanidin, pelargonidin and delphinidin (acylated anthocyanins) together with their added value due to health effects opens a new window of opportunities for the use of these pigments in a variety of food applications. The aim of this study was to extract anthocyanins from red cabbage leaves, red radish root peels and eggplant peels. And to find out the optimal levels of addition of these extracts for coloring yogurt samples and study the effect of the optimal levels of anthocyanin- rich extracts addition on some heart disease risk markers in obese aged rats.

2. MATERIALS AND METHODS

2.1. Materials: Red cabbage (*Brassica oleracea* L. var. *Capitata*), red radish (*Raphanus sativus* L.) and eggplant (*Solanum melongena* L.) were purchased from local market of Giza Governorate, Egypt. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and from common commercial suppliers.

2.2. Methods

2.2.1. Technological studies

2.2.1.1. Extraction of anthocyanins from raw materials: The leaves of red cabbage, the peels of red radish root and the peels of eggplant fruits were collected and washed in running tap water.

They were chopped into small pieces, placed in glass containers and acidified boiling water with 2% citric acid added to cover the raw materials. The raw materials were placed in a blender and blended to leach out anthocyanins. Anthocyanin rich extracts were filtered and concentrated using a laboratory rotary vacuum evaporator (RE 100 Model, Bibby Sterilin Ltd., England) rotating at 66 rpm and 40°C until the volume of the extracts reached 50% of the original volume.

2.2.1.2. Total anthocyanin contents in raw materials: Anthocyanins were expressed as mg cyanidin-3-glucoside equivalent per g fresh weight for red cabbage leaves, mg pelargonidin-3-sophoroside-5-glucoside equivalent per g fresh weight for red radish root peels and mg delphinidin-3-rutinoside equivalent per g fresh weight for eggplant peels using different molar extinction coefficients and molecular weights, since it has been reported by Giusti and Wrolstad (2001) that anthocyanins are the most dominant anthocyanin compounds in each of the three raw materials evaluated.

2.2.1.3. Preparation of yogurt samples: Yogurt was chosen to be colored with anthocyanin-rich extracts because it is a cheap product, easy to make and suitable for all ages. The process of making yogurt involves culturing milk with live and active bacterial cultures accomplished by adding bacteria directly to the milk. Commercially made yogurt is usually made with a culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus*. Yogurt samples were colored with different extracted anthocyanins using the optimal levels 2 ml (contained 1.7 mg anthocyanins); 8 ml (contained 1.8 mg anthocyanins) and 1.9 ml (contained 1.8 mg anthocyanins) of red cabbage, red radish and eggplant extracts, respectively, according to the studies performed in animals (8 mg anthocyanins / kg body weight) and humans (560 mg anthocyanins / day in adult humans) as reported by Lee *et al.* (2002) and Curtis *et al.* (2009). Half and double of these levels were used to find out the optimal levels of anthocyanin-rich extract addition. Levels of anthocyanin-rich extract used for coloring yogurt samples were: 1 ml, 2 ml and 4 ml for red cabbage; 4 ml, 8 ml and 16 ml for red radish and 0.95 ml, 1.9 ml and 3.8 ml for eggplant.

2.2.1.4. Sensory evaluation: Sensory evaluation of the different tested yogurt samples colored with different anthocyanin-rich extracts was carried out by ten trained panelists from Food Technology Research Institute, Giza, Egypt. The evaluated parameters included: Color (10), odor (10), taste (10), mouth feel (10) and appearance (10) according to Gallardo-Escamilla *et al.* (2007).

2.2.2. Biological studies

2.2.2.1. Animals and experimental diets: A total of 30 Sprague–Dawley male rats were obtained from Ophthalmology Research Institute, Giza, Egypt. Six young rats weighing 135 to 145 g were considered as young control rats and the rest of rats weighing 260 to 280 g were considered as obese aged rats. These were housed in plastic cages and fed on basal diet and water for one week (adaptation period). The basal diet was composed of casein (12%), cellulose (5%), vitamins mixture (1%), salt mixture (4%), corn oil (5%) and corn starch (73%). The basal diet formulation was performed according to A.O.A.C. (2006). The rest of rats were divided into 4 groups (n=6). The second group was fed on basal diet and considered as an obese aged control group, while the other three groups of obese aged rats were fed on basal diet and orally administered with anthocyanin-rich extracts obtained from red cabbage leaves, red radish root peels and eggplant peels at the following doses: 4 ml red cabbage anthocyanin- rich extract containing 3.4 mg cyanidin-3-glucoside or 4 ml red radish anthocyanin-rich extract containing 0.9 mg pelargonidin-3-sophoroside-5-glucoside or 3.8 ml eggplant anthocyanin-rich extract containing 3.6 mg delphinidin-3-rutinoside for each rat once daily before meals. Body weight of rats was recorded at the beginning of the study and at the end of each week. Blood samples were taken from orbital plexus venous from food–deprived rats for 12 h and the serum was separated and stored at –20°C for analysis.

2.2.2.2. Biochemical analysis: Serum total cholesterol, high-density lipoprotein cholesterol, low–density lipoprotein cholesterol, very low–density lipoprotein cholesterol and triglycerides were determined according to the methods of Roeschlau *et al.* (1974), Assmann (1979), Friedwald *et al.* (1972), Hatch and Lees (1968) and Uwajima *et al.* (1984), respectively. Serum glutathione peroxidase (GPx) and lipid peroxidation level (malondialdehyde, MDA) were estimated according to the methods described by Daret and Ching (1996) and Meltzer *et al.* (1997), respectively. Plasma glucose levels were determined according to the method of Trinder (1969). Insulin levels were measured by Ins-ELISA kit (Biosource Europe S.A., B-1400 Nivelles, Belgium) according to Temple *et al.* (1992). The homeostasis model assessment (HOMA) was used to assess insulin sensitivity index from fasting glucose and insulin concentrations. HOMA-R (insulin resistance) = glucose × insulin / 22.5, where plasma glucose is expressed in mmol/l and serum insulin is expressed in mU/L as described by Wallace *et al.* (2004). Hemoglobin concentration and blood cell counts were performed on the EDTA-treated blood according to the method of Craven *et al.* (1992). Fibrinogen levels were measured as described by Maresca *et al.* (1999). Thrombin concentrations were measured using commercial enzyme-linked immunosorbent assay according to the method of With Noto *et al.* (2008).

2.2.2.3. Statistical analysis: The standard analysis of variance procedure in a completely randomized design was applied for the present data according to Gomez and Gomez (1984). Least significant difference (LSD) test was done to compare a pair of group means. The level of statistical significance was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Technological studies

3.1.1.Total anthocyanin contents in raw materials

After the extraction of anthocyanin pigments from red cabbage leaves, red radish root peels and eggplant peels followed by concentration of the pigments using rotary evaporator, the total anthocyanin contents were determined in the three extracts and expressed as mg/g fresh weight. This is the way in which Row these crops are consumed and the way in which most references are available in the literature. It was found that red cabbage leaves contained 1.43 mg anthocyanins / g fresh weight calculated as cyanidin-3-glucoside, red radish root peels contained 0.37 mg anthocyanins / g fresh weight calculated as pelargonidin-3-sophoroside-5-glucoside and eggplant peels contained 1.60 mg anthocyanins / g fresh weight calculated as delphinidin-3-rutinoside. These anthocyanin contents are in agreement with those reported by Yuan *et al.* (2009) for red cabbage; Matera *et al.* (2012) for red radish and Thirunavukkarasu and Nithya (2011) for eggplant.

3.1.2. Sensory evaluation of yogurt samples

The data presented in Table (1) show the sensory evaluation of yogurt samples colored with different levels of anthocyanin-rich extracts from red cabbage leaves, red radish root peels and eggplant peels. It was noticed that sensory attributes, *i.e.*, appearance, color, odor, taste and mouth feel increased with increasing levels of red cabbage leaf extract. The highest scores for all attributes were also achieved by using 4 ml red cabbage anthocyanin-rich extract which was significantly different compared with the control yogurt sample for all sensory attributes except for odor. Increasing levels of red radish anthocyanin-rich extract caused significant decrease in all sensory attributes. The highest scores were observed for 4 ml red radish extract, while the lowest scores were achieved by using 16 ml red radish root anthocyanin-rich extract for all attributes. Concerning eggplant anthocyanin-rich extract, it was observed that increasing the levels of anthocyanin-rich extract caused significant increase in all sensory attributes. The highest increase was achieved by coloring yogurt samples with 3.8 ml eggplant anthocyanin extract which was significantly different compared with the control sample for all attributes except for odor. The differences among overall acceptability were more pronounced. There was a significant difference between the control yogurt sample and all other samples colored with different levels of anthocyanin-rich extracts. Yogurt samples colored

Table (1): Sensory evaluation of yogurt samples.

Yogurt samples	Appearance (10)	Color (10)	Odor (10)	Taste (10)	Mouth feel (10)	Overall acceptability(50)
Control sample	6.9±0.2^{C}	8.6±0.6^{B}	9.4±0.6^{A}	7.1±0.2^{D}	6.8±0.2^{E}	38.8±1.3^{C}
1ml red cabbage extract (a)	7.9±0.1^{B}	6.8±0.2^{D}	7.9±0.1^{B}	6.6±0.1^{E}	7.7±0.4^{C}	36.9±1.1^{D}
2ml red cabbage extract	8.1±0.5^{B}	7.8±0.4^{C}	9.0±0.6^{A}	8.2±0.6^{B}	8.0±0.4^{C}	41.1±1.3^{B}
4ml red cabbage extract	9.3±0.8^{A}	9.4±0.5^{A}	9.1±0.7^{A}	8.4±0.6^{B}	8.7±0.6^{B}	44.9±1.4^{A}
4ml red radish extract (b)	9.1±0.6^{A}	9.8±0.7^{A}	9.0±0.5^{A}	8.7±0.3^{A}	7.9±0.5^{C}	44.5±1.8^{A}
8ml red radish extract	6.8±0.3^{C}	7.2±0.3^{C}	8.5±0.4^{AB}	7.1±0.4^{D}	7.3±0.6^{D}	36.9±1.2^{D}
16ml red radish extract	5.9±0.1^{D}	6.5±0.2^{D}	6.3±0.2^{D}	6.4±0.1^{E}	6.7±0.3^{E}	31.8±1.1^{F}
0.95ml eggplant extract (c)	6.7±0.2^{C}	7.3±0.4^{C}	7.0±0.3^{C}	6.5±0.3^{E}	7.0±0.2^{ED}	34.5±1.3^{E}
1.9ml eggplant extract	9.0±0.6^{A}	7.5±0.2^{C}	8.8±0.4^{AB}	7.7±0.2^{C}	8.7±0.5^{B}	41.7±1.5^{B}
3.8ml eggplant extract	9.3±0.9^{A}	9.2±0.8^{A}	9.5±0.6^{A}	8.7±0.5^{A}	9.1±0.7^{A}	45.8±1.7^{A}
L.S.D.	0.38	0.63	0.57	0.26	0.36	1.36

a = 1 ml red cabbage extract contained 0.85 mg anthocyanin calculated as cyanidin-3-glucoside.
b = 4 ml red radish extract contained 0.90 mg anthocyanin calculated as pelargonidin-3-glucoside.
c = 0.95 ml eggplant extract contained 0.96 mg anthocyanin calculated as delphinidin-3-rutinoside.
*Values in the column with different letters are significantly different at $p < 0.05$.

with 4 ml red cabbage anthocyanin-rich extract (contained 3.4 mg anthocyanins), 4 ml red radish anthocyanin-rich extract (contained 0.9 mg anthocyanins) or 3.8 ml eggplant anthocyanin-rich extract (contained 3.6 mg anthocyanins) were not significantly different compared with each other and resulted in the highest scores for overall acceptability compared with other samples. From Table (1) it could be concluded that, the optimal levels of anthocyanin rich extracts for coloring yogurt were 4 ml (contained 3.4 mg anthocyanins), 4 ml (contained 0.9 mg anthocyanins) and 3.8 ml (contained 3.6 mg anthocyanins) from red cabbage leaves, red radish root peels and eggplant peels, respectively.

3.2. Biological studies

3.2.1. Effect of anthocyanin-rich extracts on body weight in obese aged rats

The data presented in Table (2) show the effect of anthocyanin-rich extract administration from red cabbage, red radish and eggplant on body weight in obese aged rats at zero time, after 15 days, 21 days and at the end of the experiment (30 days). Body weight of young control rats did not significantly change during the experimental period, but it was significantly different compared with the body weight of obese aged control rats at zero time and also during the experimental period. Body weight of obese aged control rat group did not significantly change during the experimental period. There was a significant gradual decrease in body weight for all anthocyanin-rich extract groups from zero time to the end of the experiment. The reduction in body weight was 16.98, 18.63 and 19.91% for rats orally administered with red cabbage, red radish and eggplant anthocyanin rich extracts, respectively, compared with obese aged control rats at the end of the experiment. The highest reduction in body weight was achieved by using 3.8 ml eggplant anthocyanin extract containing 3.6 mg anthocyanin followed by 4 ml red radish anthocyanin extract containing 0.9 mg anthocyanin.

Obesity is a strong risk factor for hypertension, hyperlipidemia, heart disease and type 2 diabetes. It was found that mice fed the high fat diet plus purified anthocyanins from blueberries in the drinking water had lower body weight gains and body fat than the high fat fed controls. Anthocyanins fed as the whole blueberry did not prevent, and may have actually increased obesity. However, administration purified anthocyanins from blueberries or strawberries reduced obesity (Luke *et al.* 2008). Anthocyanin fraction significantly increased the phosphorylation of adenosine monophosphate–activated protein kinase (AMPK) and acetyl-coenzyme A carboxylase (ACC) in the liver, suggesting a potential target for the prevention of obesity (Hwang *et al.,* 2011 and Wu *et al.,* 2013). Consumption of purified mulberry anthocyanins can significantly inhibit body weight gain, lower the size of adipocytes, attenuate lipid accumulation and decrease leptin secretion. Thus, dietary supplementation with anthocyanins can protect body weight gain of the diet-induced obese mice.

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3.2.2. Effect of anthocyanin-rich extracts on glucose homeostasis in obese aged rats

Blood glucose levels, serum insulin levels and insulin resistance calculated by homeostasis model assessment index as affected by

Table (2): Effect of anthocyanin rich extracts on body weight in obese aged rats.

Time / Groups	Body weight (g)			
	At zero time	**After 15 days**	**After 21 days**	**At the end**
Young control rats	136.16±3.55^{E}	138.63±3.41^{E}	142.30±2.65^{E}	144.66±2.47^{E}
Obese aged control rats	271.64±5.42^{A}	269.43±5.43^{A}	264.70±5.34^{A}	261.52±4.65^{A}
4 ml red cabbage anthocyanin extract (a)	269.35±5.33^{A}	253.62±4.68^{B}	228.40±2.35^{C}	217.10±1.69^{D}
4 ml red radish anthocyanin extract (b)	270.55±4.72^{A}	256.30±4.61^{B}	229.63±2.47^{C}	212.80±1.81^{D}
3.8 ml eggplant anthocyanin extract (c)	266.77±5.25^{A}	249.60±3.92^{B}	226.19±1.68^{C}	209.45±1.72^{D}
L.S.D.	19.53	8.41	6.74	17.80

a = 4 ml red cabbage extract contained 3.4 mg anthocyanin calculated as cyanidin-3-glucoside.
b = 4 ml red radish extract contained 0.90 mg anthocyanin calculated as pelargonidin-3-glucoside.
c = 3.8 ml eggplant extract contained 3.6 mg anthocyanin calculated as delphinidin-3-rutinoside.
L.S.D. for interaction between time and treatments = 10.56
*Values in the column with different letters are significantly different at $p < 0.05$.

anthocyanin-rich extracts are presented in Table (3). Blood glucose level was significantly increased by 1.32 fold in obese aged control rats compared with young control rats at the end of the experimental period. Blood glucose level in obese aged rats orally administered with red cabbage, red radish and eggplant anthocyanin -rich extracts significantly decreased compared with obese aged control rats, but they not significantly different compared with young control rats and between each other. The decrease in blood glucose levels as a result of administration with different anthocyanin rich extracts were 23.73, 24.89 and 27.93% for red cabbage, red radish and eggplant anthocyanin rich extracts, respectively. Serum insulin level of obese aged control rats was significantly higher compared with young control rats and all other groups at the end of the experiment. Administration of obese aged rats on different anthocyanin-rich extracts resulted in a significant decrease in serum insulin levels. Red cabbage and red radish anthocyanin-rich extract groups were not significantly different compared with each other and with young control rats, but they were significantly different compared with eggplant anthocyanin-rich extract group. The reduction in insulin levels was 30.90, 29.20 and 35.62% for red cabbage, red radish and eggplant anthocyanin-rich extract, respectively. The highest reduction in blood glucose and serum insulin levels was achieved by using 3.8 ml eggplant anthocyanin-rich extract containing 3.6 mg anthocyanin calculated as delphinidin-3-rutinoside. It could be observed from Table (3) that, insulin resistance was significantly increased in obese aged control rats compared with young control rats and all other experimental groups. Insulin resistance index (HOMA-R) was significantly decreased in anthocyanin-rich extract groups compared with obese aged control rats group. Red cabbage and red radish anthocyanin-rich extract groups were not significantly different compared with young control rat group and between each other, but they were significantly different when compared with eggplant anthocyanin rich extract group which caused the highest reduction in insulin resistance index.

Cardiovascular disease in patients with diabetes is clearly associated with the degree of hyperglycemia. Anthocyanins improve insulin sensitivity and glucose uptake in diabetic rats and suggesting that they could be successfully used as insulin–sensitizing agents (Prior *et al.,* 2010). Dietary bilberry anthocyanin extract significantly reduced the blood glucose concentration and enhanced insulin sensitivity through activation of adenosine monophosphate–activated protein kinase (AMPK). This activation was accompanied by up-regulation of glucose transporter 4 (GLUT4) in white adipose tissue and skeletal muscle and suppression of glucose production and lipid content in the liver. The same authors also showed that cyanidin-3-glucoside and its metabolite, protocatechuic acid, increased adipocyte glucose uptake and glucose transporter 4 membrane translocation, which indicate that cyanidin-3-glucoside and protocatechuic acid might exert insulin-like activities. An anthocyanin-rich berry extract supplemented to an unbalanced diet normalized insulin resistance and insulin concentration was ameliorated to levels comparable with the rats fed control diet (Takikawa *et al.*, 2010 and Adam *et al.,* 2013).

3.2.3. Effect of anthocyanin-rich extracts on oxidative status in obese aged rats

The data presented in Fig. (1) showed the effect of anthocyanin-rich extracts on malondialdehyde (nmol/ml) and glutathione peroxidase(U/ml) in obese aged rats. It was found that, malondialdehyde levels (MDA) significantly increased in obese aged control rat group compared with young control rat group and all other groups at the end of the experimental period. Administration of anthocyanin-rich extracts caused significant reduction in MDA levels of obese aged rats compared with obese aged control

Table (3): Effect of anthocyanin rich extracts on glucose homeostasis in obese aged rats.

Groups \ Time	Glucose (mmol/L)		Insulin (mU/L)		HOMA-R
	Zero	End	Zero	End	
Young control rats	5.13±0.62^{C}	5.22±0.46^{C}	33.06±1.12^{C}	33.51±1.11^{C}	7.77±0.64^{B}
Obese aged control rats	5.84±0.57^{B}	6.91±0.54^{A}	36.18±1.10^{B}	47.64±1.26^{A}	14.63±0.94^{A}
4 ml red cabbage anthocyanin extract(a)	5.65±0.55^{B}	5.27±0.60^{C}	35.63±0.95^{B}	32.92±0.97^{C}	7.71±0.60^{B}
4 ml red radish anthocyanin extract (b)	5.80±0.60^{B}	5.19±0.63^{C}	36.44±1.16^{B}	33.73±1.06^{C}	7.78±0.72^{B}
3.8 ml eggplant anthocyanin extract(c)	5.77±0.54^{B}	4.98±0.51^{C}	37.16±1.24^{B}	30.67±1.08^{D}	6.79±0.55^{C}
L.S.D.	0.48		2.11		0.63

a = 4 ml red cabbage extract contained 3.4 mg anthocyanin calculated as cyanidin-3-glucoside.
b = 4 ml red radish extract contained 0.90 mg anthocyanin calculated as pelargonidin-3-glucoside.
c = 3.8 ml eggplant extract contained 3.6 mg anthocyanin calculated as delphinidin-3-rutinoside.
*Values in the column with different letters are significantly different at $p < 0.05$.

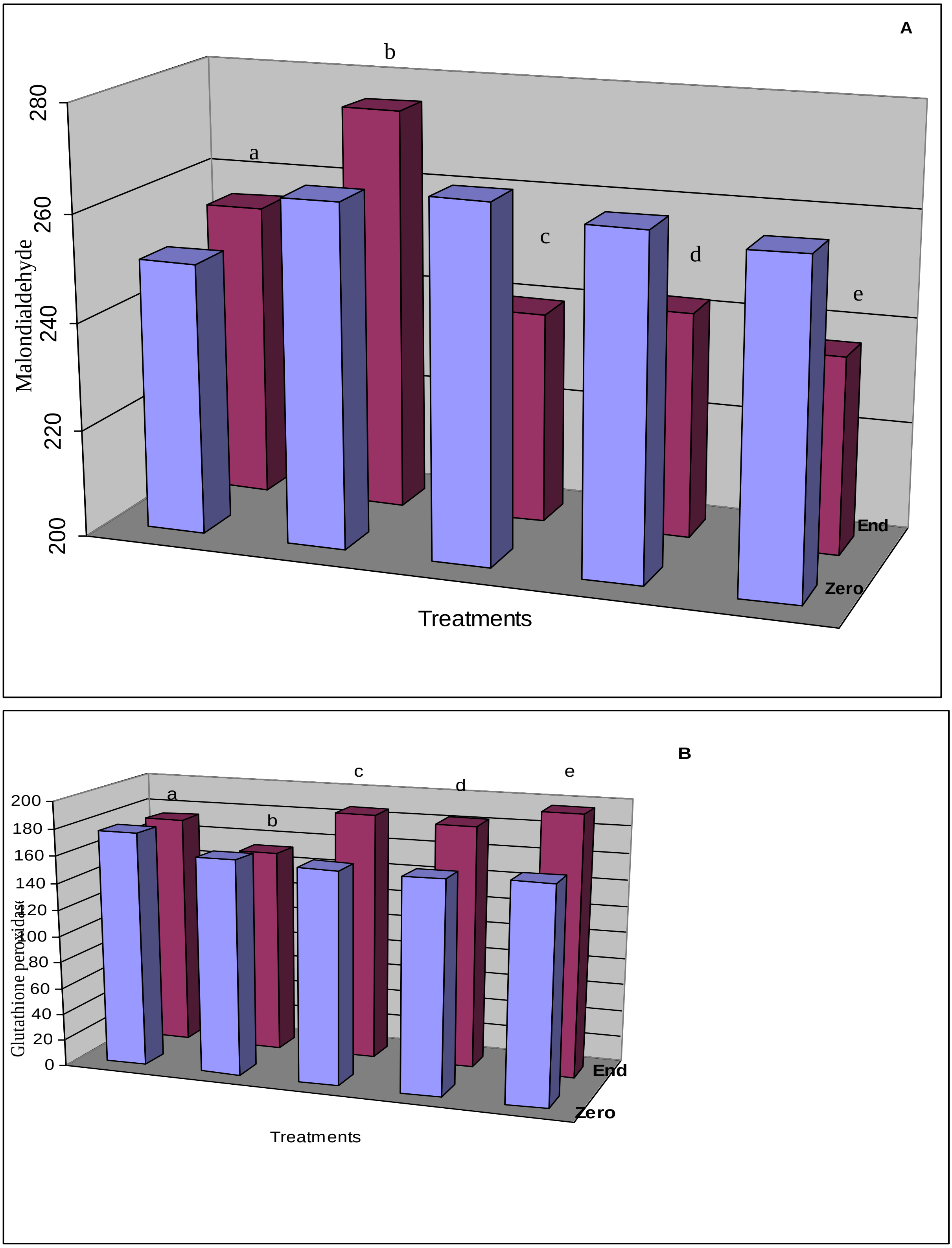


Fig. (1): Effect of anthocyanin-rich extracts on malondialdehyde (A) and glutathione peroxidase (B) in obese aged rats. a= Young control rats, b= Obese aged control rats, c= Red cabbage anthocyanin-rich extract, d= Red radish anthocyanin-rich extract and e= Eggplant anthocyanin-rich extract.

and young control rats. The highest reduction in MDA levels was achieved by using 3.8 ml eggplant anthocyanin-rich extract (14.08%) containing 3.6 mg anthocyanin calculated as delphinidin-3-rutinoside followed by 4 ml red cabbage anthocyanin-rich extract (13.03%) containing 3.4 mg anthocyanin calculated as cyaniding-3-glucoside and 4 ml red radish anthocyanin-rich extract (12.08%) containing 0.9 mg anthocyanin calculated as pelargonidin-3-glucoside. Anthocyanin-rich extract groups were not significantly different compared with each other at the end of the experiment for MDA levels. Concerning glutathione peroxidase levels (GPx), it could be noticed that the administration of obese aged rats on different anthocyanin-rich extracts caused significant increase in GPx enzyme activity compared with obese aged control rats which was significantly decreased at the end of the study period. GPx level for obese aged control rats was significantly lower compared with young control rats. The increase in GPx activity of orally administered anthocyanin -rich extracts was as follows: eggplant > red cabbage > red radish.

The role of anthocyanins in CVD prevention is strongly linked to the protection against oxidative stress. The concentration of anthocyanins in the serum was directly correlated to the serum antioxidant capacity when adult males were supplemented with 1.2 g of anthocyanins from freeze-dried blueberries. This change in antioxidant capacity suggests that anthocyanins and their decretory products may play an important role in decreasing the production of superoxide and other mechanisms which can lead to an increase in serum antioxidant capacity. Delphinidin which is found in eggplant peels was the most potent anthocyanidin in the suppression of oxidative stress-induced endothelial injury (Mazza *et al.* 2002 and Shilpa and Tucker, 2011). It was found that, blueberries anthocyanin may improve metabolic syndrome and related cardiovascular risk factors and decreases plasma oxidized LDL and serum malondialdehyde concentrations than in the control group. Also, treatment with anthocyanins prevented the formation of hepatic MDA and the depletion of glutathione activity in the livers of intoxicated rats (Basu *et al.*, 2010).

In mice, glutathione peroxidase deficiency results in abnormal vascular and cardiac function and structure. Dietary anthocyanins modulate cardiac antioxidant defenses. Rats treated with anthocyanin-rich extract showed a better profile of the antioxidant system with normal glutathione peroxidase activity. Delphinidin imposed preventive effects on suppressing the production of lipid peroxidation and restoring the activities of endogenous antioxidants. Anthocyanins (cyanidin fraction) showed protective effects *in vitro* against oxidative stress in a dose–dependent manner and had significantly higher free radical–scavenging capacity than the non-anthocyanin fraction (Chen *et al.*, 2010 and Im *et al.*, 2013).

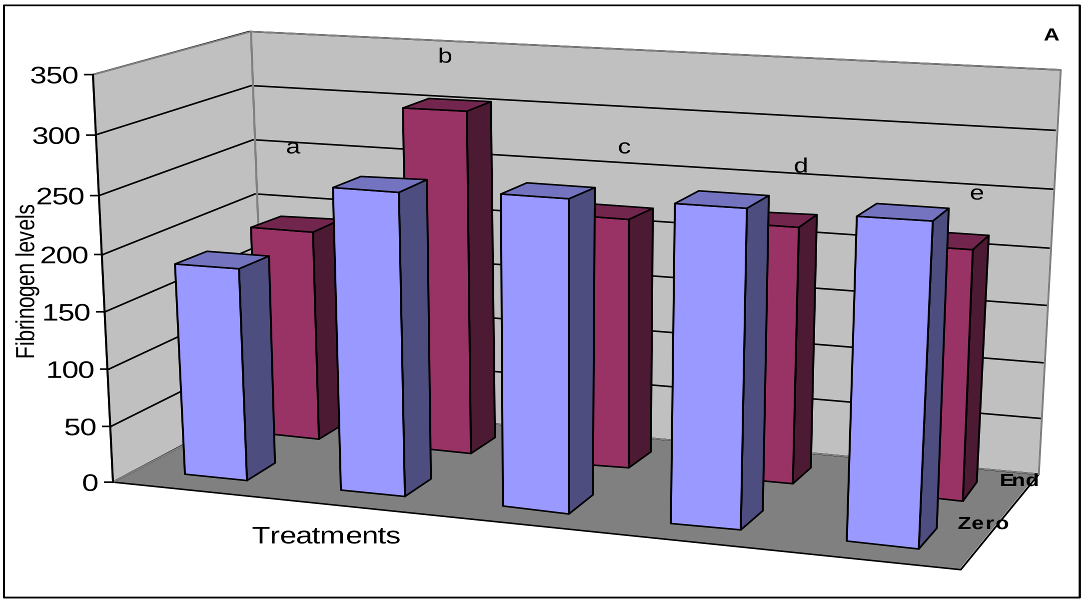
3.2.4. Effect of anthocyanin-rich extracts on hemoglobin concentrations, White blood cell counts and platelet counts in obese aged rats

Hemoglobin concentrations, white blood cell counts and platelet counts were significantly low in obese aged control rats at zero time and at the end of the experimental period compared with young control rats (Table 4). White blood cell counts in obese aged control rats at the end of the experiment were significantly lower compared with zero time. Oral administration of anthocyanin-rich extracts in obese aged rats caused significant increase in hemoglobin concentrations, white blood cell counts and platelet counts when compared with obese aged control rats at the end of the experiment. Obese aged rats fed on red cabbage and red radish anthocyanin-rich extracts were not significantly different compared with young control rats for hemoglobin concentrations and platelets counts at the end of the experimental period. Eggplant anthocyanin-rich extract caused a significant increase in platelet counts compared with other groups. The highest increase in hemoglobin concentrations, white blood cell counts and platelet counts was achieved by using 3.8 ml eggplant anthocyanin-rich extract containing 3.6 mg delphinidin-3-rutinoside followed by 4 ml red cabbage anthocyanin-rich extract containing cyaniding-3-glucoside and 4 ml red radish anthocyanin-rich extract containing 0.9 mg pelargonidin-3-glucoside, respectively.

Platelets are essential for primary hemostasis and repair of the endothelium. Chronic cranberry juice consumption (containing high levels of anthocyanins) has high bioavailability profiles and rapid access to the cells in the circulation, including platelet, at concentrations potentially capable of eliciting modulatory effects (Dohadwala *et al.*, 2011).

3.2.5. Effect of anthocyanin-rich extracts on fibrinogen levels and thrombin concentrations in obese aged rats

The data presented in Fig. (2) showed the effect of anthocyanin-rich extract administration on fibrinogen levels and thrombin concentrations in obese aged rats. Fibrinogen levels increased by1.6 fold in obese aged control rats compared with young control rats at the end of the experimental period. They were significantly changed compared with that of both young control and obese aged rat groups at zero time and at the end of the experiment. Fibrinogen levels in obese



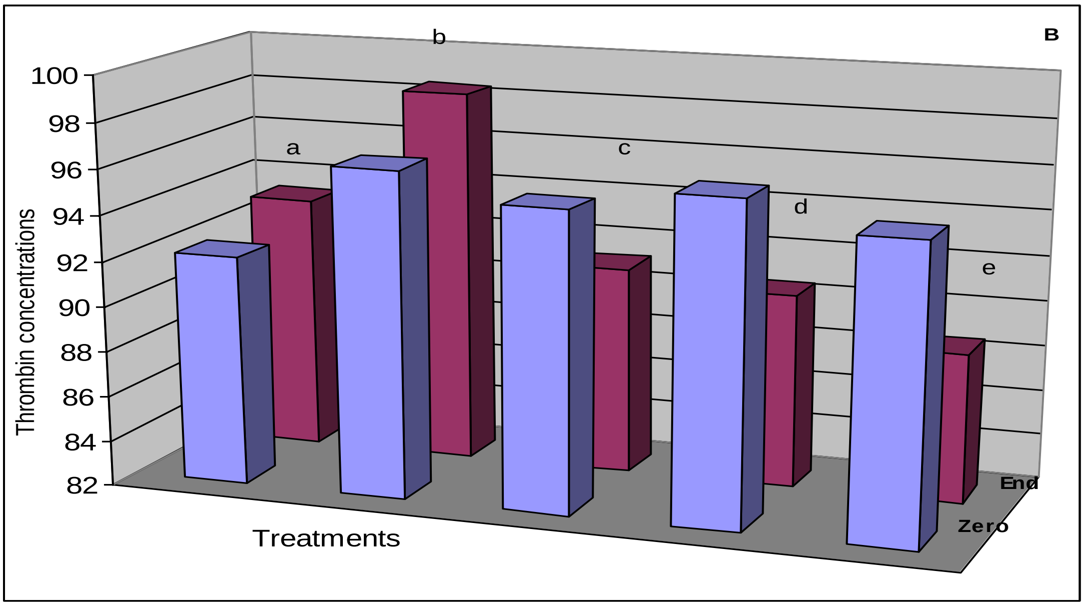


Fig. (2): Effect of anthocyanin-rich extracts on fibrinogen levels (A) and thrombin concentrations (B) in obese aged rats. a= Young control rats, b= Obese aged control rats, c= Red cabbage anthocyanin-rich extract, d= Red radish anthocyanin-rich extract and e= Eggplant anthocyanin-rich extract.

aged rats orally administered with anthocyanin-rich extracts were significantly decreased than those of obese aged control rats both at zero time and at the end of the experimental period. Fibrinogen levels for anthocyanin-rich extract groups were not significantly different compared with each other. The decrease in fibrinogen levels as a result of the treatments was 27.82, 26.78 and 29.44% for red cabbage, red radish and eggplant anthocyanin-rich extracts, respectively. From Fig. (2) it could be observed that, thrombin concentrations were significantly increased in obese aged control rats compared with young control rats and the increase was significantly different at the end of the experimental period. Thrombin concentrations had the same trend as fibrinogen levels by the administration of different anthocyanin-rich extracts which caused significant reduction in thrombin compared with obese aged control rats both at zero time and at the end of the experiment. Thrombin concentrations for red cabbage and red radish anthocyanin-rich extract groups were not significantly different compared with each other and young control rats, while eggplant anthocyanin-rich extract group showed a statistically significant difference compared with other groups. The highest decrease in thrombin concentration was achieved by using eggplant followed by red radish and red cabbage anthocyanin rich extracts, respectively.

Fibrinogen is a circulating glycoprotein that acts at the final step in the coagulation response to vascular and tissue injury. Fibrinogen levels tend to be higher in patients with diabetes, Hypertension, obesity and those with sedentary lifestyles. Hepatic synthesis of fibrinogen can increase up to 4 folds in response to inflammatory or infectious triggers. In clinical studies, there have been strong associations between increased plasma fibrinogen levels and the risk of cardiovascular disease which suggests hyperfibrinogenemia as an independent predictor of vascular events. Cardiovascular disease outcome would be prevented by adding information on fibrinogen to the standard risk factors used to predict the risk of cardiovascular event (Green *et al.,* 2010 and Borissoff *et al.,* 2012). Thrombin is a unique serine protease that is pivotal to coagulation and that may also display various actions toward other systems (*e.g.,* immune, nervous, gastrointestinal and musculoskeletal systems). Thrombin may potentiate atherogenic processes, such as endothelial dysfunction, oxidative stress, inflammation and activation of platelets and leukocytes, which suggests an important role in the pathogenesis of cardiovascular disease. Protocatechuic acid (PCA) has an antithrombotic effect which was confirmed *in vivo* in a rat arterial thrombosis model, where PCA significantly delayed the arterial occlusion without increasing bleeding risks (Borissoff *et al.,* 2009 and Milburry *et al.,* 2012).

3.2.6. Effect of anthocyanin-rich extracts on lipid profiles in obese aged rats

Data in Table (5) showed the effect of anthocyanin-rich extracts on lipid parameters measured in the serum of obese aged rats. Obesity resulted in a significant increase in serum triglycerides levels compared with young control rats. Serum triglycerides level was significantly increased in obese aged control rats compared with young control rats and all other groups at the end of the experimental period. Feeding obese aged rats with different anthocyanin-rich extracts showed a significant reduction in triglyceride levels compared with obese aged control rats at the end of the experiment. There was no statistical significant difference between red cabbage, red radish and eggplant anthocyanin-rich extracts for triglyceride levels at the end of the study period. From the data in Table (5) it could be observed that the total cholesterol, LDL-cholesterol, and VLDL-cholesterol levels were significantly increased in obese aged rats compared with young control rats both at zero time and at the end of the experimental period. Oral administration of anthocyanin-rich extracts in obese aged rats caused significant reduction in the levels of lipid profiles (total cholesterol, LDL and VLDL-cholesterol) compared with obese aged control rats. The highest reduction in lipid profiles was achieved by using 3.8 ml eggplant anthocyanin-rich extract containing 3.6 mg delphinidin-3-rutinoside. The reduction in triglycerides, total cholesterol, LDL and VLDL-cholesterol levels was not significantly different compared with young control rats for red cabbage, red radish and eggplant anthocyanin-rich extract groups. On the other side, HDL-cholesterol level significantly decreased in obese aged control rats compared with young control rats. Feeding obese aged rats with different anthocyanin-rich extracts caused a significant increase in the levels of HDL–cholesterol compared with both obese aged control and young control rats at zero time and at the end of the experimental period. The highest increase in HDL-cholesterol was achieved by using eggplant, red radish and red cabbage anthocyanin rich extracts, respectively.

In hypercholesterolemic individuals, endothelial function can be impaired by dyslipidemia and mild chronic inflammation represented as increased total cholesterol or LDL-cholesterol concentrations. Supplementation with diets rich in anthocyanins or pure anthocyanin-rich extracts resulted in a decrease in serum triglyceride, total cholesterol and LDL-cholesterol and an increase in serum HDL-cholesterol in different animal models. This prompted to suggest that pure anthocyanins may result in a greater reduction in CVD risk factors (Rogers and Fuke, 2006).

Hypolipidemic effects of mulberry anthocyanin extract are *via* an enhancement of LDL-R gene expression and the clearance ability of LDL and a decrease in the lipid biosynthesis (Duchnowicz *et al.,* 2012). It has been reported that the cardiovascular disease event rate is reduced by nearly 1% for each 1% reduction in LDL and by >1% for each 1% increase in HDL. Therefore, the decrease in LDL-cholesterol and the increase in HDL-cholesterol observed in the present study due to the administration of anthocyanin rich extracts would result in a reduction in heart disease risk, which is meaningful and greatly

Table (4): Effect of anthocyanin-rich extracts on hemoglobin concentrations, white blood cell counts and platelet counts in obese aged rats.

Time / Groups	Hemoglobin concentrations		White blood cells counts		Platelet counts	
	Zero	**End**	**Zero**	**End**	**Zero**	**End**
Young control rats	12.46± 0.64^{B}	12.87± 0.65^{B}	9340.66± 74.93^{A}	9600.33± 94.21^{A}	640.35± 17.33^{B}	676.42± 15.62^{B}
Obese aged control rats	11.93± 0.43^{C}	11.55± 0.40^{C}	6600.00± 69.55^{D}	5930.15± 52.33^{E}	470.36± 11.52^{C}	445.60± 10.47^{C}
4 ml red cabbage (a)	11.82± 0.47^{C}	13.28± 0.71^{AB}	6430.74± 64.35^{D}	8370.66± 66.84^{B}	465.74± 10.68^{C}	683.20± 14.32^{B}
4 ml red radish (b)	11.68± 0.48^{C}	13.17± 0.76^{AB}	6500.00± 69.40^{D}	7200.17± 99.20^{C}	456.18± 11.64^{C}	655.63± 13.20^{B}
3.8 ml eggplant (c)	11.90± 0.39^{C}	13.46± 0.77^{A}	6566.64± 60.55^{D}	8650.14± 100.25^{B}	460.86± 11.44^{C}	744.15± 10.44^{A}
L.S.D.	0.47		466.57		53.07	

a = 4 ml red cabbage extract contained 3.4 mg anthocyanin calculated as cyanidin-3-glucoside.
b = 4 ml red radish extract contained 0.90 mg anthocyanin calculated as pelargonidin-3-glucoside.
c = 3.8 ml eggplant extract contained 3.6 mg anthocyanin calculated as delphinidin-3-rutinoside.
***Values in the column with different letters are significantly different at $p < 0.05$.**

Table (5): Effect of anthocyanin-rich extracts on lipid profiles in obese aged rats (mg/dl).

Time / Groups	Triglycerides		Total cholesterol		HDL-cholesterol		LDL-cholesterol		VLDL-cholesterol	
	Zero	**End**	**Zero**	**End**	**Zero**	**End**	**Zero**	**End**	**Zero**	**End**
Young control rats	90.17± 2.42^{C}	93.31± 1.84^{C}	80.33± 1.33^{C}	85.60± 1.67^{C}	38.19± 0.92^{C}	39.07± 0.81^{C}	24.11± 0.10^{C}	27.87± 0.15^{C}	18.03± 0.09^{C}	18.66± 0.04^{C}
Obese aged control rats	103.63± 2.35^{B}	115.20± 3.41^{A}	107.64± 2.04^{B}	135.56± 3.55^{A}	35.25± 0.54^{D}	28.63± 0.26^{E}	51.66± 0.14^{B}	83.89± 1.05^{A}	20.73± 0.13^{B}	23.04± 0.02^{A}
4ml red cabbage(a)	105.18± 2.51^{B}	93.10± 1.09^{C}	115.86± 3.05^{B}	85.72± 1.08 ^{C}	36.54± 0.66^{D}	41.29± 0.47^{B}	58.28± 0.18^{B}	25.81± 0.63^{C}	21.04± 0.06^{B}	18.62± 0.06^{C}
4ml red radish (b)	106.34± 2.40^{B}	90.84± 1.06^{C}	120.13± 3.64^{B}	90.84± 1.12^{C}	35.31± 0.30^{D}	43.60± 0.50^{A}	63.55± 0.21^{B}	29.07± 0.16^{C}	21.27± 0.11^{B}	18.17± 0.09^{C}
3.8ml eggplant (c)	104.45± 2.60^{B}	90.60± 1.11^{C}	112.50± 2.46^{B}	83.66± 1.44^{C}	36.42± 0.67^{D}	44.36± 0.41^{A}	55.19± 0.26^{B}	21.18± 0.10^{C}	20.89± 0.07^{B}	18.12± 0.08^{C}
L.S.D.	4.17		13.45		6.56		12.05		0.65	

a = 4 ml red cabbage extract contained 3.4 mg anthocyanin calculated as cyanidin-3-glucoside.
b = 4 ml red radish extract contained 0.90 mg anthocyanin calculated as pelargonidin-3-glucoside.
c = 3.8 ml eggplant extract contained 3.6 mg anthocyanin calculated as delphinidin-3-rutinoside.
***Values in the column with different letters are significantly different at $p < 0.05$.**

promising. Anthocyanin prevents or reverses hypercholesterolemia – induced endothelial dysfunction by inhibiting cholesterol and 7-oxysterol accumulation in the aorta of mice fed the anthocyanin compared with mice fed high cholesterol diet. The cholesterol oxidation product, 7-ketocholesterol, increased to 180.1 µg/ml in the control emulsion, while it was only 15.4 and 39.0 µg/ml in the emulsions containing 1 µg/ml of the anthocyanin and tocol extracts, respectively, according to Wang *et al.* (2012a) and Zhang *et al.* (2013), who also reported that anthocyanin extract was relatively greater than tocol extract in stabilizing cholesterol.

Increasing HDL-cholesterol levels has been considered as an important strategy for preventing and treating CVD. In addition to its anti-oxidative and anti-inflammatory properties, HDL may attenuate atherosclerosis *via* stimulating reverse cholesterol transport (RCT). It is a process that entails the efflux of excess cholesterol from macrophages into the liver followed by its excretion into the bile and then the feces. Protocatechuic acid (PCA) promotes cholesterol transportation of HDL from intestinal tissue into gut lumen or reduces intestinal cholesterol and bile acid re-absorption, thus partially contributing to PCA-induced macrophage RCT (Wang *et al.,* 2012b).

3.2.7. Effect of anthocyanin-rich extracts on total cholesterol, HDL and LDL–cholesterol ratios in obese aged rats

The data presented in Fig. (3) showed the effect of anthocyanin-rich extract administration on ratios of total:HDL-cholesterol, LDL:HDL-cholesterol and HDL:LDL–cholesterol in obese aged rats. It was found that ratios of total:HDL-cholesterol and LDL:HDL-cholesterol were significantly higher in obese aged control rats compared with young control rats at the end of the experimental period, while the reverse was true for the ratio of HDL:LDL-cholesterol which was higher in young control rats. Administration of anthocyanin rich extracts caused a significant reduction in the ratios of total:HDL-cholesterol and LDL:HDL-cholesterol compared with obese aged control rats. The highest reduction in both ratios was achieved by using eggplant anthocyanin-rich extract. The ratio of HDL:LDL-cholesterol showed significant increase after administration of anthocyanin-rich extracts compared with obese aged control rats and the highest increase was recorded for eggplant followed by red cabbage and red radish anthocyanin rich extracts, respectively.

Total: HDL-cholesterol ratio is more sensitive and specific than the total cholesterol as a risk predictor for CVD. A change of 1 U in total:HDL-cholesterol is associated with a 53% change in the risk of CVD and a weight loss of 3 kg may lead to a decrease of 0.24 U in total:HDL-cholesterol, which underlines the importance of weight management in the reduction of CVD risk (Assmann *et al.,* 1996). Each increase of 1 mg/dl (0.03 mmol/l) in HDL-cholesterol is associated with a decrease of 2 to 3% in the risk of future heart disease. Intervention trials using LDL-cholesterol have consistently shown substantial reductions in major cardiovascular events with reduction of LDL levels and each decrease of 40 mg/dl (1.0 mmol/l) in LDL-cholesterol corresponding to a 24% reduction in major cardiovascular events. The ratio of LDL:HDL-cholesterol was highly predictive of major cardiovascular events. Subjects with the highest ratios of LDL:HDL-cholesterol and total:HDL-cholesterol had a significantly greater risk of major cardiovascular events than did subjects with the lowest ratios (Nicholls *et al.* 2008).

In conclusion, anthocyanin rich extracts have a potential benefit as an alternative to synthetic dyes for coloring yogurt. The best samples were made by using 3.8 ml eggplant, 4 ml red cabbage and 4 ml red radish anthocyanin-rich extracts corresponding to 3.6 mg, 3.4 mg and 0.9 mg of the major anthocyanin component in each case, respectively. Administration of anthocyanin-rich extracts to obese aged rats caused a significant reduction in established risk markers of heart disease including body weight, blood glucose, serum insulin, insulin resistance index, MDA levels, fibrinogen levels, thrombin concentrations, triglycerides, lipid profiles, ratios of total:HDL-cholesterol and LDL:HDL-cholesterol. A significant increase in GPx activity, hemoglobin, white blood cells counts, platelet counts, HDL-cholesterol and HDL:LDL-cholesterol ratio was achieved by using anthocyanin rich extracts. Administration of 3.8 ml eggplant anthocyanin-rich extract containing 3.6 mg delphinidin-3-rutinoside as the main anthocyanidin resulted in an improvement in most of the above risk markers compared with other anthocyanin-rich extracts. Thus, it is recommended to use anthocyanin-rich extracts for coloring different food products to obtain their healthy benefits, keep in mind the suitable amount of each extract according to human body weight and body mass index.

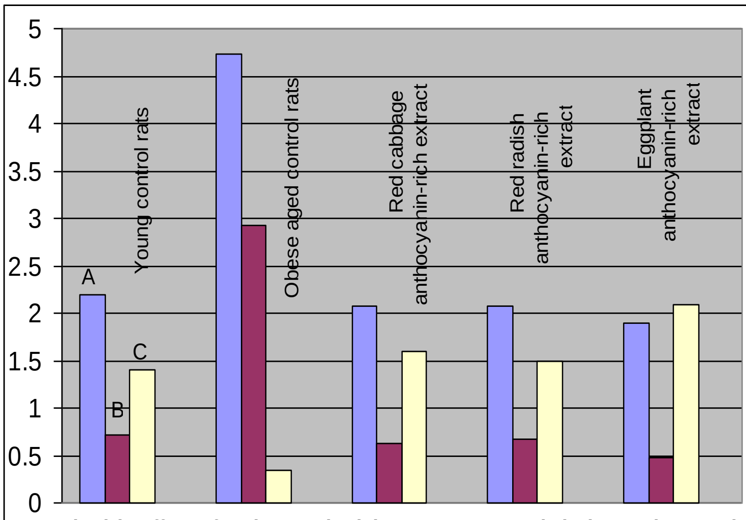


Fig. (3): Effect of anthocyanin rich extracts on total cholesterol, HDL-cholesterol and LDL-cholesterol ratios in obese aged rats. A = Total:HDL-cholesterol ratio, B = LDL:HDL-cholesterol ratio and C = HDL:LDL- cholesterol ratio.

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تاثير الخضراوات الغنيه بالأنثوسيانين على عوامل الخطر لأمراض القلب

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ملخص

مرض القلب هوالسبب الرئيسى للوفاة فى العالم . يعتبر منع الاصابة به على قمة الأهتمامات الصحية، وجد أن الأغذيه والمشروبات الغنية بالأنثوسيانين تحمى تركيب الدهون وتحسن العديد من عوامل الخطر لأمراض القلب من خلال خواصها كمضادات للألتهابات ومضادات للأكسدة. ولذلك فأن الغرض من هذا البحث هو: استخلاص الأنثوسيانينات من أوراق الكرنب الاحمر، قشور جذور الفجل الاحمر، قشور الباذنجان بتركيزات مختلفة، ثم ايجاد المعدل الامثل للأضافة لتلوين عينات الزبادى ودراسة تاثير اضافة مستخلصات الأنثوسيانين على عوامل الخطر لأمراض القلب فى الفئران كبيرة السن الزائده فى الوزن لتقليل أو تحسين عوامل الخطر. وقد وجد من خلال هذه الدراسه أن اوراق الكرنب الاحمر وقشور جذور الفجل الاحمر وقشور الباذنجان تحتوى على 1.43 ملجم، 0.37 ملجم، 1.60 ملجم من الأنثوسيانين لكل جم من الوزن الرطب، على التوالى وأن أفضل عينات الزبادى من وجهة نظرالمستهلك كانت باضافة 3.8 مل من مستخلص قشور الباذنجان الغنى بالأنثوسيانين، 4 مل مستخلص اوراق الكرنب الاحمر الغنى بالأنثوسيانين، 4 مل مستخلص قشور جزورالفجل الاحمر الغنى بالأنثوسيانين المحتوى على 3.6 ملجم، 3.4 ملجم، 0.9 ملجم انثوسيانين على التوالى بالمقارنة مع باقى العينات.

وجد من خلال هذه الدراسه أن مستخلصات الأنثوسيانين أدت لخفض معنوى فى الوزن للفئران كبيرة السن الزائده فى الوزن، وقد وجد أن مستويات الجلوكوز فى الدم والأنسولين فى السيرم أظهرت انخفاضاً معنوياً بعد اضافة مستخلصات الأنثوسيانين للفئران كبيرة السن والزائده فى الوزن بالمقارنة مع الفئران الكنترول كبيرة السن الزائده فى الوزن. وقد ظهر انخفاض معنوى فى مستوى أكسدة الدهون (المالونداى الدهيد) وزيادة معنوية فى نشاط انزيم الجلوتاثيون بيروكسيديز بتقديم مستخلصات الأنثوسيانين. انخفضت معنويا مستويات الفيبرينوجين فى الفئران كبيرة السن الزائده فى الوزن والتى حصلت على مستخلصات الأنثوسيانين عن طريق الفم بالمقارنة مع مجموعة الكنترول وقد ظهر أن تركيزات الثرومبين اتخذت نفس المسار مثل مستويات الفيبرينوجين، وقد وجد أن معاملة الفئران كبيرة السن الزائده فى الوزن بمختلف مستخلصات الأنثوسيانين أظهرت تحسن معنوى فى مستويات الجليسريدات الثلاثيه، الكوليستيرول الكلى، الكوليستيرول منخفض وشديد الأنخفاض فى الكثافة، الكوليستيرول عالى الكثافة بالمقارنة مع مجموعة الكنترول فى نهاية فترة التجربة.

من النتائج المتحصل عليها من هذه الدراسه يمكن استنتاج أن مستخلصات الأنثوسيانين من مختلف الأجزاء النباتية يمكن استخدامها كبديل لاستخدام الصبغات الصناعية لتلوين المنتجات الغذائيه كما أن لها فوائد صحية مهمة خاصة لمعالجة وتحسين عوامل الخطر لأمراض القلب وغيرها من الأمراض المرتبطة بالتقدم فى السن.

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