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**Antioxidant Activities of Betalains Extracts Add to
Doughnut During Storage and the Role of Red Beet
Ingestion on Anemia in Rats**

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ABSTRACT

The present study was carried out to evaluate the concentration of betalain, in red beet root by adding betalain extract to doughnut as a source of natural colorants, food additives, natural antioxidants and study the role of red beet ingestion on anemia in rats. Chemical composition, some mineral, total phenol, flavonoid contents and total betalains of red beet was determined. Also chemical composition of doughnut made from wheat flour with betalain extract fresh and after storage for 4 weeks was determined.

Evaluation of antioxidant activity on lipids extracted from doughnut as measured by using acids and peroxide values at zero time and after storage 4 week. Microbial count of doughnut during storage 4 weeks was determined. On the other hand study the role of red beet ingestion on anemia in rats. This study was conducted on twenty albino male rats classified into four groups (n=5). The first group kept as normal group (control -ve), other three groups administered a single dose of phenyl hydrazine injection 40 mg/kg/day for two days, then rats classified into three groups one as a control positive (+ve) (untreated) and two groups treated with red beet juice at ratio 2 and 5ml respectively by stomaic tube daily. The study assigned for four weeks.

The results showed that red beet has high levels of P, K, Mg and Ca values were 87.8, 681.3, 57.9 and 44.7 mg/100 g. In addition, it contains Fe (2.12) and Se (1.66) mg/100 g on dry weight. The results recorded that total phenol was (879.3) and flavonoid was (223.4) mg/100g on dry weight. It contained betalain 352.8mg/100g on wet weight. the results revealed that peroxide value was decreased in

doughnut treated with betalain extracts 5ml and 2ml. The results showed significant increase in hemoglobin noticed in rats groups treated with red beet 2 and 5ml which reached 10.9 and 11.7g/dl respectively. Also significant increase in RBCs was noticed of rats groups treated with red beet root 2 and 5ml. The results showed significant decrease in serum triglyceride, total cholesterol and LDL-c, VLDL-c levels and a significant increase in HDL-c level was observed in rats treated with red beet compared with positive control.

Keywords: Betalains, Antioxidant , Total phenol, Hemoglobin ,Malonaldehyde

1.INTRODUCTION

Beta Vulgaris, Amaranthaceae family. It used for jaundice, inflammatory and anemia, Beet root good for hypertension due to its high content of potassium, It is very important for neonatal due to its content of iron and folic acid, also help to get rid of fat in case of obesity and atherosclerosis. (El- Kolla, 2013).

Red beet (*Beta vulgaris L.*) includes specifically high amount of antioxidants. Red beet contains many phenolic compounds such as conjugates phenolic amides, ferulic acid and flavonoids, Also contain betalains, which are natural food colorants, (composed of betaxanthins and betacyanins) (Wootten, *et al.*, 2011 and Wootten *et al.*, 2011).

Red beet is an excellent source of Ascorbic acid, Thiamine, betalain, folates, Fe, Mg, P and K. It is beneficial for digestive problems, skeletal system and circulatory system. (Alina and Camelia 2013). They supply the principle antioxidants which help to scavenge the free radicals. They have several therapeutic properties like immune boosting; treating anemia, circulatory disorders, cancer, and alcohol induced liver failure, digestive disorders. Beet root as vegetable are consumed raw as well as cooked.(Mighana, *et al.*, 2013)

Beet root contains a large amount of pigments such as betaxanthins and betacyanin of the betalain family, most studies indicated to important of betalains as anti-oxidative stress, anti-inflammation and antitumor (Ninfali and Angelino 2013).

Beet root pigment is used as a food additive. It changes colour when heated, it is cheap and has no known allergic side-effects. (NRCS, 2006).

Natural colorants in fruits and vegetables have disadvantages as higher cost and reduced stability. Betalains pigments, found in red beet (*Beta vulgaris*). Betalains consist of betacyanins (red-violet pigments) and betaxanthins (yellow-orange pigments) (Delgado Vargas *et al.*, 2000; Herbach *et al.*, 2004, Stintzing & Carle, 2004). Anthocyanins widely spread and used as natural pigments, betalains are more stable to pH and temperature. (Stintzing & Carle, 2004).

The most significant betalain compound is betanin, which is a glycoside composed of glucose and betanidin. Beet root has a red and purple color due to betacyanin and betanin. (Wootton, *et al.*, 2011a, and Wootton *et al.*, 2011b).

The attention paid to compounds showing high antioxidant activity, such as carotenoids, anthocyanins and recently betalains has increased (Frank *et al.*, 2005). Beetroot, delivered acutely as a juice supplement (Webb *et al.*, 2008 & Bailey, *et al.*, 2009 and Jajja *et al.*, 2014) or in bread (Hobbs *et al.*, 2012 & Hobbs *et al.*, 2013).

Anemia is one of the oldest blood malformation, it is a blood cells disorder. Anemia arises because of curtailed formation of erythrocytes and it cannot function like normal one and have short lifespan. (Zidison 1994).

Phenylhydrazine decreases red blood cell, haemoglobin level, packed cell volume, and impairs erythrocyte deformability. It increased osmotic resistance, free plasma haemoglobin, and extramedular haematopoiesis in the spleen and liver (Cira and Ogawa, 1975 and Mstern 1999).

The objective of the present study evaluated the red beet which has a high concentration of betalains, by adding betalains extract to doughnut as a source of natural colorants, food additives and natural antioxidants and study the role of red beet ingestion on anemia in rats.

2.MATERIAL AND METHODS

2.1 Materials:

2.1.1 Red beet root: was obtained from farmer of El-Senblaween, El - Mansoura, Egypt.

2.1.2 Wheat flour 72% extraction (*Triticum aestivum L*): was obtained from the North Flour Mills Company, Egypt.

2.1.3 Shortening : (Rawaby as refined palm oil, 100% pure vegetables oil and cholesterol free) was obtained from Safola Egypt Company 10th

Ramadan City, Cairo. Skim milk, vanilla, sugar, eggs and baking powder were purchased from local market in Mansoura city.

2.1.4 Rats: Normal male albino rats (110 ±15g) were obtained from Helwan Station, Ministry of Health and Population, Cairo, Egypt.

2.1.5 Chemicals: Casein, as main source of protein from Morgan Company for Chemicals, Cairo, Egypt and vitamins and salts mixtures, all organic solvents and other chemicals were of analytical grade were purchased from El-Ghomhoria Company for Drugs, Cairo, Egypt.

2.2 Methods:

2.2.1 Preparation of betalains extraction.

Betalains was extracted according to. (Kavitha *et al.*, 2013).

2.2.2 Preparation of Doughnut :

Doughnut was prepared using 100 gm wheat flour (72%), sugar 11.4g, shortening 9.4 g, whole egg 20g, skim milk 54g, baking powder 1.5g, salt 1.5g, roll butter cold 75.2g, vanilla 5g. Other formulation using amounts of betalains extracts.

2.2.3 Determination of chemical composition .

Proximate chemical composition of samples (Moisture, protein, total fat, ash and crude fiber) were determined according to the methods of the A.O.A.C. (2000), carbohydrates estimated by difference as following:

Carbohydrates% = 100 - (% moisture + % protein + % fat +%ash).

2.2.4 Determination of some minerals .

Some minerals content calcium (Ca), magnesium (Mg), potassium (K), phosphor (P), iron (Fe) and selenium (Se) were determined in the diluted solution of ash of raw materials and their blends using the atomic absorption spectrophotometer (3300 Perkin-Elme) as described by AOAC (2000) method.

2.2.5 Determination of total phenolic compounds.

Total phenolics were determined by HPLC according to the method of Goupy *et al.*, (1999) at Central lab. of Food Technology Research Institute, Agric. Res. Cent. Egypt .

2.2.6 Determination of flavonoid.

Total flavonoid was determined by HPLC according to the method of Merfort, *et al.* (1997).

2.2.7 Determination of total betalains:

The concentrated red beet was determined by Castellar *et al.*, (2003)

2.3 Evaluation of antioxidant activity for doughnut .

2.3.1 Acid and peroxide values :

Acid and peroxide values were determined in each sample according to the method described by (AOAC 2000).

2.2.5 Determined of total counts of bacteria.

Total counts of bacteria (nutrient agar media), yeast and mold were determined (potato dextrose agar media) according the methods of (BAM, 1998).

2.2.3 Experimental diets

The basic diet prepared according to AIN, (1993) and Reeves *et al.*(1993) as following: protein, corn oil and vitamin mixture. (10%), (10%) and (1%), mineral mixture, choline chloride, methionine and cellulose (4%), (0.2%), (0.3%) and (5%) and the remained is corn starch (69.5%). The used vitamin mixture component was that recommended by (Campbell, 1963) while the salt mixture used was formulated according to (Hegsted, 1941).

2.2.4 Experimental animal design.

The experimental rats were fed on basal diet for five days before starting the experiment for adaptation, then the rats were allocated into four equal groups. Normal control group fed on the basal only (n=5) ,while the other three groups were administered a single dose of phenyl hydrazine injection 40 mg/kg/day according to Josef Berger, (2007) for two day, then rats classified into three groups which were control positive (+ve) (untreated) and two groups treated with red beet juice 2 and 5ml by stomach tube daily. The study assigned for four weeks. The feed intake was calculated daily and the body weight gain was recorded weekly. At the end of experiment food efficiency ratio was calculated as following:

$$\text{FER} = \frac{\text{Body weight gain (gm)}}{\text{Food intake (gm)}}$$

2.2.3.1 The analytical methods of blood serum:

Blood samples were collected. Heparinized blood to estimat hemoglobin (HB), red blood cells (RBCs), white blood cells (WBCs) and platelets count (PLT), asses by Drabkin (1949) and Mc Inory (1954), respectively.

Total antioxidants and superoxide dismutase enzymes (SOD) were determined by Habig (1974), Beuchamp and Fridovich, (1971) respectively.

Tc, HDL-c and TG content were determined by enzymatic colorimetric method according to (Allian *et al.*, 1974, Richmond 1973 and Fossati and Principle 1982), respectively. LDL-cholesterol and VLDL-cholesterol were calculated by the (Friedewald *and* Fredrickson 1972).

Serum (ALT, AST), were estimated giving by Reitman and Frankel (1957), while alkaline phosphatase (ALP) , total bilirubin and total protein determined by (Kind and King 1954), Hare (1950) and Fossati, *et al.*, (1980), respectively.

Statistical analysis:

Results of the biochemical estimations of the rats are reported as mean \pm S.E.M. (Standard Error of Mean). The total variation was analysed by performing one-way analysis of variance. "LSD (Least Significant Difference) test" was used for determining significance (Sümbüloglu *et al.* 1998). Probability levels of less than 0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Chemical composition and some minerals of red beet root samples.

The proximate composition revealed that red beet contained 73.25g moisture, 19.67 g carbohydrates, 13.12 g fiber, 3.81 g protein, 2.18g ash and 1.09 g fat per 100 g on wet weight basis (Table 1). These results are accordance with that of (Meghana *et al.*, 2013), who stated that red beet is a good source of dietary fiber and has practically little fat. Red beet roots is a good source of fiber, colorful and very few calories, it does not contain fat. (Compendium of Beet Diseases and Insects, 1986).

The results in Table (1), showed that red beet has high levels of P, K, Mg and Ca (87.8, 681.3, 57.9 and Ca 44.7mg/100g respectively. In addition, it contains Fe (2.12) and Se (1.66) mg/100g on dry weight .These results indicate that beet root contain Se which are important for antioxidant enzymes in vivo and hence protect the body from cancer. In addition, beet root could be considered a good food supplement because it contains amount of K, P ,Mg , Ca, Fe and Se.

These results are in accordance with that of (Yashwant ,2015) who found that beet root rich in calcium, magnesium, phosphorus, potassium, and sodium. Also, smaller amounts of iron, zinc, copper, manganese, and selenium.

Table (1): Chemical composition, some minerals contents of red beet root

Chemical composition g/100g (wet weight basis)		Mineral content mg/100g (dry weight basis)	
Moisture	73.25	Ca	44.7
Protein	3.81	Mg	57.9
Fat	1.09	K	681.3
Ash	2.18	P	87.8
Carbohydrates	19.67	Fe	2.12
Crude fiber	13.12	Se	1.66

Results expressed as mean values (n= 3).

3.2 Total phenol, flavonoid contents and total betalains in red beet root

Total phenol and flavonoid were determined in red beet their concentrations were registered in Table (2), the results showed that total phenol was (879.3) and flavonoid was (223.4) mg/100g on (DW). flavonoids, and phenols increase antioxidant and biological activities of extracted betalain. (Wootton *et al.*, 2014 ; Cruz-Cansino *et al.*, 2015 and Sravan Kumar *et al.* , 2015). Epicatechin, rutin, and caffeic acid antioxidants in beet root identified in betalain family. (Georgiev *et al.*, 2011). Beet root is a good source of antioxidant attributable to total phenolic content 50–60 $\mu\text{mol/g}$ dry weight (Vinson *et al.*, 1998; Kahkonen *et al.*, 1999). Total betalains was determined in red beet, their concentrations were registered in Table (1), the results showed that total betalains was 352.8352.8 mg/100 on (Ww). The results obtained in general were in accordance with (Zakharova and Petrova 1997), who found that, the total betalain content of red beet were 250 mg/100g to 850 on fresh weight while (Delgado *et.al.*, 2000) found that, red pigment content in red beet could reach 500 mg /100g on fresh weight. These results are coinciding with that of (Wybraniec, 2005), who mentioned that the major components of pigments in red beet are betalain and isobetalain.

Table (2): Total phenol, Flavonoid contents and Total betalains in red beet root

Variables	mg/100g
Total Phenol	879.3
Flavonoid	223.4
Total betalains	352.8

3.3 Chemical composition of doughnut made from wheat flour with betalains extracts fresh and after storage for 4 weeks.

The results in Table (3) showed the chemical composition of doughnut made from wheat flour with betalains extracts ,the results revealed that moisture has high content in doughnut treated with betalains extracts 5ml which recorded 28.4 g/100g and after storage which recorded 24.54g/100g followed by doughnut treated with betalains extracts 2ml which recorded 25.78 and after storage recorded 21.3 than control which recorded 24.0 and after storage which recorded 13.90. The results showed that doughnut treated with betalains extracts 5ml was higher content of protein which recorded 7.16 and after storage which recorded 5.80 followed by doughnut treated with betalains extracts 2ml which recorded 6.82 and after storage which recorded 5.25 than control which recorded 6.23 and after storage which recorded 4.90. Also, in the same Table doughnut treated with betalains extracts 5ml has higher content in fats which recorded 33.5 and after storage which 28.10 followed by doughnut treated with betalains extracts 2ml which recorded 32.00 and after storage which recorded 34.6 than control which recorded 30.00 and after storage 34.30. Results shows that high fat in control or doughnuts treated with betalains extract 2ml, this is due to the moisture decrease after storage 4 week, on the other hand fat decrease in doughnut treated with betalains extract 5ml, may be due to the moisture was high and natural antioxidants were effective in maintaining a low oxidation level in doughnut during storage 4 week. Betalains showed high antioxidant and anti-inflammatory capabilities in vitro and a variety of in vivo animal models (Zielińska *et al.*, 2009 and Vulić, *et al.*, 2014). Color is important indicator to accept foods. Application of synthetic colorants has decreased in favor of natural colorants (Kavitha *et al.*, 2013). Beet root cultivated in many countries, consumed as part of

normal diet, and as a food colouring agent known as E162 (Zielińska *et al.*, 2009 and Tom, *et al.*, 2015).

Table (3): Chemical composition of doughnut made from wheat flour with betalains extracts fresh and after storage for 4 weeks

Samples	Control	Doughnut with 2ml	Doughnut with 5ml
Moisture			
Zero time	24.0	25.78	28.4
After storage	13.90	21.3	24.54
Protein			
Zero time	6.23	6.82	7.16
After storage	4.90	5.25	5.80
Fat			
Zero time	30.00	32.00	33.5
After storage	34.30	34.6	28.10
Ash			
Zero time	0.94	1.05	1.31
After storage	1.45	1.5	1.40
Carbohydrates			
Zero time	38.83	34.89	29.63
After storage	45.45	37.35	40.16

3.4 Inhibitory effect of betalains extract on the primary oxidation of lipids extracted from doughnut as measured by using acids and peroxide values.

Data in Table (4) recorded the inhibitory effect of betalains extracts on the primary oxidation of lipids extracted from doughnut as measured by acid and peroxide values after storage 4 week, the results showed that acid value was decreased in doughnut treated with betalains extracts 5ml which recorded 2.54 mg KOH/gm followed by doughnut treated with betalains extract 2ml which recorded 2.85 mg KOH/g than control which recorded 3.48 mg KOH/g after storage 4 week. Also, the results revealed that peroxide value was decreased in doughnut treated with betalains extracts 5ml followed by 2ml which recorded 4.18 and 5.25 meq/kg than control which recorded 7.87 meq/kg after storage 4 week. Beetroot is known to be a powerful antioxidant (Christiana *et al.* , 2005). The betalains, sugars, terpenoids, phenolics, and ascorbic acid in

beet root are known to reduce lipid peroxidation (Rice-Evans and Miller 1996 and Yang *et al.*.,2001 and Váli *et al.*, 2007).

The pigments in root contains oxalic acid, ascorbic acid, betalains, and phenolics, showed a potent antioxidant activity based on its radical scavenging activity (DPPH). (Wettasinghe *et al.*, 2002 and Krajka *et al.*., 2013). Beet root pigments consists of two main groups, the red betacyanins and the yellow betaxanthins. They are free radical scavengers (Pedreno and Escibano, 2001).

Therefore, betalains can be used as a ingredient added to food products as good source of natural colorants, food additives and natural antioxidants in the human diet. Natural antioxidants in betalains were effective in maintaining a low oxidation level in doughnut during storage 4 week.

Table (4): Inhibitory effects of betalains extracts on lipids extracted from doughnut as measured by acids and peroxide values during storage 4 week

Samples	Control	Doughnut with 2ml	Doughnut with 5ml
Acid value			
Zero time	1.74	1.70	1.44
After storage	3.48	2.85	2.54
Peroxide value			
Zero time	3.77	3.85	3.16
After storage	7.87	5.25	4.18

3.5 Effect of storage on microbiological analysis of doughnut made from wheat flour with betalains extracts at room temperature

Data in Table (5), showed the microbial quantity inhabited in doughnut treated with 5 and 2ml betalain extract after storage 4 week. Microbial quantity decreased significantly in doughnut treated with betalains extracts 5ml which recorded 31.67 ± 2.517 Cfug 10^2 followed by doughnut treated with betalains extracts 2ml which recorded 36.67 ± 0.577 Cfug 10^2 than control which recorded 62.67 ± 7.024 Cfug 10^2 after storage three week .The present results showed that betalains extracts has high capacity to inhibit microbial count. These results are accordance with that of (Fontanet *et al.*, 2013), who found that beet root juice and throughout storage time is antimicrobial. Betalain has

antioxidant and antimicrobial activities. (Kathiravan, *et al.*, 2014) and. (Kujaka *et al.*, 2000). Betanines have antioxidant, antimicrobial and antiviral activity, antimicrobial activity showed on *Staphylococcus aureus* and *Escherichia coli* (Prahoveanu *et al.*, 1986, Rauha *et al.*, 2000 and Pedreno and Escribano, 2001).

Table (5): Effect of storage on microbiological analysis of doughnut made from wheat flour with betalains extracts after storage at room temperature (Cfu/g 10²)

Total count	Control	Doughnut with 2ml	Doughnut with 5ml
Zero time	1.33 a ±0.577	1.67 a ±0.577	1.60 a ±0.35
After one week	15.00 a ±4.163	10.63 ab ±1.528	9.00 ab ±1.732
After two week	36.33 a ±6.110	23.33 c ±4.726	20.67 c ±3.786
After three week	62.67 a ±7.024	36.67 c ±0.577	31.67 c ±2.517
After four week	Un accounted	42.67b ±2.517	41.67 b ±2.517

*a, b and c means in the same row with different superscripts are different significantly (P< 0.05).

3.6 Effects of red beet root on BWG, FI and FER of experimental rats

Data in Table (6) showed significant increase in the body weight gain (BWG), food intake (FI) and food efficiency ratio (FER) of all rat groups treated with red beet than positive control. The results showed that an increase significantly in BWG was noticed in rats group treated with red beet 2ml which reached 63.43(g) and rats group treated with red beet 5ml which reached 66.65(g) than positive control which reached 41.88(g). Also the results revealed that an increase significantly in FI was recorded in rats group treated with red beet 2ml which reached 15.99 (g/day) and rats group treated with red beet root 5ml which reached 16.21 (g/day) when compared with positive control which reached 11.73(g/day). On the same table, an increase in FER was observed in groups treated with red beet 2 and 5ml which reached 0.142 and 0.147 respectively than positive control which reached 0.126. Díaz-

Castro *et al.*,(2008) showed significant decrease in the body weights of the anemic rats.

Table (6): Effects of red beet on BWG, FI and FER of experimental rats

Groups	Negative control	Positive control	Red beet 2ml	Red beet 5ml
B W G (g)	95.72±8.42a	41.88±5.1 d	63.43±6.13 c	66.65±5.57 c
FI (g/day)	16.85±1.84a	11.73±1.17 c	15.99±1.45 b	16.21±1.22 a
FER	0.203±0.001a	0.126±0.003d	0.142±0.005c	0.147±0.004 b

*a, b and c means in the same row with different superscripts are different significantly (P< 0.05).

3.7 Effects of red beet on hemoglobin, RBCs, WBCs and PLT of experimental rats.

As evident from Table (7) a significant increase in hemoglobin, red blood cells (RBCs) and significant decrease in white blood cells (WBCs) and platelets count (PLT) of groups treated with red beet than positive control. The results showed that significant increase in hemoglobin was noticed of rats groups treated with red beet 2 and 5ml which reached 10.9 and 11.7 g/dl respectively than positive control which reached 7.77 g/dl. Also the results showed significant increase in RBCs was noticed of rats groups treated with red beet 2 and 5ml which reached 4.7 and 4.9 10⁶/ml respectively when compared to positive control which reached 2.5810⁶/ml On the other hand, results recorded a significant decrease in WBCs and PLT was noticed in rats treated with 2ml red beet which reached 5.8 and 317 10³/ml respectively, and rats treated with 5ml red beet which reached 4.85 and 336.25 respectively, than positive control which showed 7.6 and 391 10³/ml respectively. These results advised that, it is important to give red beet juice to the patients suffering of anemia. These results are accordance with that of (Benkovic *et al.*, 2009) who showed that beet root mainly stimulates the factors related to RBCs rather than WBCs.

Table (7): Effect of red beet on hemoglobin, RBCs, WBCs and PLT of experimental rats.

Groups	Negative control	Positive control	Red beet 2ml	Red beet 5ml
Hemoglobin(g/dl)	12.12±1.2a	7.77±0.22d	10.9±0.3c	11.7±2.4b
RBCs 10 ⁶ /ml	6.4±0.21b	2.58±0.2e	4.7±1.1d	4.9±0.9d
WBCs 10 ³ /ml	4.4±1.4c	7.6±1.3a	5.8±0.2b	4.85±0.3c
PLT 10 ³ /ml	293±16.8f	391±7.4a	317±8.3e	336.25±18.4d

*a, b and c means in the same row with different superscripts are different significantly (P< 0.05).

3.8 Effects of red beet on some antioxidant parameters of experimental rats.

Data in Table (8) revealed significant increase in total antioxidants (TA), superoxide dismutase (SOD), which observed in rats group treated with red beet than positive control. An increase in TA was observed in rats group treated with 2ml red beet reached to 3.32 mmol/L and rats group treated with 5ml red beet reached to 3.94 mmol/L when compared to positive control which reached 1.67 mmol/L. Also the results showed that an increase in SOD in rats group treated with red beet 2ml and 5ml which reached 54.14 and 59.87U/l respectively when compared with positive control which showed 21.25 (U/l). A relation between content of red pigments and antioxidant capacity of red beet was studied, red betanin is primarily responsible for the antioxidant activity. (Czapski *et al.*, 2009). Red beet juice showed stronger antioxidant properties (Walkowiak-Tomczak and Zielińska 2006). Beetroot has plenty of betalains that are related to the protection against oxidative stress and inflammation (Ninfali & Angelino 2013).

Table (8): Effects of red beet on some antioxidant parameters of experimental rats.

Groups	Negative control	Positive control	Red beet 2ml	Red beet 5ml
Total antioxidants mmol/L	4.55 ± 0.22 a	1.67 ± 0.15 c	3.32 ± 0.15 b	3.94 ± 0.06 b
Superoxide dismutase(U/l)	65.13 ± 5.22 a	21.25 ± 3.47d	54.14 ± 7.16 c	59.87 ± 6.35 b

*a, b and c means in the same row with different superscripts are different significantly (P< 0.05).

3.9 Effects of red beet on lipid parameters of experimental rats

The results in Table (9) showed the significant decrease in serum triglyceride, total cholesterol LDL-c, VLDL-c levels and a significant increase in HDL-c level were observed in rats treated with red beet than positive control. Data present in Table (9) revealed that a significant decrease in total cholesterol was observed of rats treated with red beet 2ml which reached 110.67 ± 7.26 (mg/dl) and rats group treated with red beet 5ml which reached 97.80 ± 2.44 (mg/dl) when compared with positive control which reached 128.57 ± 4.71 (mg/dl). Also in the same table, the results showed that a significant decrease in triglyceride was recorded of rats treated with red beet 2ml which reached 89.60 ± 3.14 (mg/dl) and rats group treated with red beet 5ml which reached 74.47 ± 5.22 (mg/dl) when compared with positive control which reached 99.13 ± 2.97 (mg/dl). On the other hand, the results in Table (9) showed that increase significantly in HDL-c was observed of rats treated with red beet 2ml which reached 31.17 ± 1.76 (mg/dl) and rats group treated with red beet 5ml which reached 33.53 ± 3.02 (mg/dl) when compared with positive control which reached 28.23 ± 1.37 (mg/dl). The results showed that decrease significantly in LDL-c was observed of rats treated with red beet 2ml which reached 61.58 ± 5.76 (mg/dl) and rats group treated with red beet 5ml which reached 49.37 ± 1.34 (mg/dl) when compared with positive control which reached 80.51 ± 3.55 (mg/dl). These results are accordance with that of (Wootton, *et al.*, 2011a and Wootton *et al.*, 2011b) who showed that betacyanin can decrease LDL cholesterol oxidation and is effective on cardiovascular diseases. Also in Table (9) the results showed that a significant decrease in VLDL-c was noticed of rats treated with red beet 2ml which reached 17.92 ± 0.63 (mg/dl) and rats group treated with red beet 5ml which reached 14.89 ± 1.04 (mg/dl) when compared with positive control which reached 19.83 ± 2.13 (mg/dl). As a powerful antioxidant pigment, betanin may provide protection and reduce risk of cardiovascular disease (Rakin *et al.*, 2007). Red beet juice improves brain function and reduces blood pressure and the occurrence of cardiovascular diseases (Ahluwalia 2010; Presley *et al.* 2010). Beetroot significantly reduce systolic and diastolic blood pressure. beetroot's anti-hypertensive potential (Hobbs *et al.* , 2013, Lidder and Webb 2013 & Apil *et al.*, 2014 &). Beetroot's effect on the vasculature is largely attributed to its high inorganic nitrate content (250

mg·kg⁻¹) of fresh weight (Ormsbee *et al.*, 2013). Red beetroot had betalains and phenolic compounds which prevent oxidation of low density lipoproteins (LDL) and prevent cardiovascular diseases. (Singh, and Hathan, 2014 and Burcu *et al.*, 2016). So advice to give red beet root juice to patients with hyperlipidemia or atherosclerosis.

Table (9): Effects of red beet on lipid parameters of experimental rats

Groups	Negative control	Positive control	Red beet 2ml	Red beet 5ml
TC (mg/dl)	82.27±4.16d	128.57±4.71a	110.67±7.26 b	97.80±2.44 c
TG (mg/dl)	68.13±2.97d	99.13±2.97 d	89.60±3.14 b	74.47±5.22bc
HDL-c (mg/dl)	39.50±2.29a	28.23±1.37c	31.17±1.76b	33.53±3.02b
LDL-c (mg/dl)	29.14±5.99e	80.51±3.55a	61.58±5.76b	49.38±1.34c
VLDL-c (mg/dl)	13.63±0.59d	19.83±2.13a	17.92±0.63b	14.89±1.04bc

*a, b and c means in the same row with different superscripts are different significantly (P< 0.05).

3.10 Effects of red beet on liver function of experimental rats

As evident from Table (10) significant decrease in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin and increase significantly in total protein were observed in the groups treated with red beet root when compared with positive control. Data present in Table (10) showed that a significant decrease in AST, were observed of rats group treated with red beet 2ml which reached 49.37±6.01 (μ /l) and rats group treated with red beet 5ml which reached 46.14±8.1(μ /l) when compared to positive control which reached 67.39±9.61(μ /l). Also in the same table, the results showed that a significant decrease in ALT, were recorded of rats group treated with red beet 2ml which reached 15.61±1.81 (μ /l) and rats group treated with red beet 5 ml which reached 14.78±2.01 (μ /l) when compared to positive control which reached 25.55±3.35(μ /l).

Concerning ALP data presented in Table (10) revealed significant decrease of rats treated with red beet 2ml which reached 36.80±4.11 (μ /l) and rats group treated with red beet 5ml which reached 34.13±4.37 (μ /l) when compared with positive control which reached

50.38±5.81(μ /l). Results in Table (10), indicated that significant decrease in total bilirubin were recorded in rats group treated with red beet 2ml which reached 0.99±0.02 (mg/dl) and rats group treated with red beet 5ml which reached 0.88±0.12 (mg/dl) when compared with positive control which reached 1.82±0.11(mg/dl).

On the other hand, the results in Table (10) showed that increase significantly in total protein was noticed of rats treated with red beet 2ml which reached 5.51±0.81(g/dl) and rats group treated with red beet 5ml which reached 6.61±0.77(g/dl) when compared with positive control which reached 4.41±1.01(g/dl). Beet root was used before Christ long ago, Medical personals reported their medical applications in liver inflammation and fatty liver (El- Kolla, 2013). Beet root juice may help to lower blood pressure and protect liver damage when included in the diet (Coles and Clifton 2012).

Table (10): Effects of red beet on liver function of experimental rats

Groups	Negative control	Positive control	Red beet 2ml	Red beet 5ml
AST(μ /l)	43.17±5.81 ^d	67.39±9.61 ^a	49.37±6.01 ^c	46.14±8.1 ^b
ALT(μ /l)	13.35±1.12 ^d	25.55±3.35 ^a	15.61±1.81 ^c	14.78±2.01 ^b
ALP (μ /l)	32.17±5.66 ^d	50.38±5.81 ^a	36.80±4.11 ^c	34.13±4.37 ^b
Total bilirubin(mg/dl)	0.72±0.01 ^d	1.82±0.11 ^a	0.99±0.02 ^b	0.88±0.12 ^c
Total protein(g/dl)	6.53±0.26 ^a	4.41±1.01 ^d	5.51±0.81 ^c	6.61±0.77 ^b

*a, b and c means in the same row with different superscripts are different significantly (P< 0.05).

CONCLUSION

Therefore, betalains can be used as ingredient added to food products as a good source of natural colorants, food additives and natural antioxidants in the human diet. Natural antioxidants in betalains were effective in maintaining a low oxidation level in doughnut during 4 week storage. Further works are needed to better understand the mechanisms to take advantage of beet root in food preparation and food industry. Beet root improve anemia and affects cardiovascular system, due to its high content of betalains ,minerals, antioxidants and high biological activity.

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فعالية الأنشطة المضادة للأكسدة لمستخلص البيتاين المضاف إلى الدونات أثناء التخزين ودور تناول عصير البنجر الأحمر على فقر الدم في الفئران لبنى احمد شلبايه ، امانى احمد سلو كلية التربية النوعية جامعة المنصورة

الملخص العربي

الهدف من هذه الدراسة تقييم جذور البنجر الأحمر الذي يحتوي على نسبة عالية من البيتاين، وذلك بإضافة مستخلص البيتاين للدونات كمصدر للأصبغ الطبيعية، والمضافات الغذائية ومضادات الأكسدة الطبيعية ودراسة دور تناول البنجر الأحمر على فقر الدم في الفئران. اولا تم تحديد التركيب الكيميائي، وبعض المعادن، الفينولات الكلييه، و الفلافونيد و البيتاين الكلي في البنجر الأحمر. أيضا تم تحديد التركيب الكيميائي للدونات المصنوعة من دقيق القمح مع مستخلص البيتاين قبل وبعد التخزين لمدة ٤ أسابيع. تم تقييم النشاط المضاد للأكسدة للدهون المستخلصة من الدونات باستخدام رقم الحموضة ورقم البيروكسيد خلال فترة التخزين ٤ أسابيع. أيضا تم تحديد العد الميكروبي للدونات خلال فترة التخزين ٤ أسابيع. من ناحية أخرى دراسة دور تناول البنجر الأحمر على فقر الدم في الفئران. وقد أجريت هذه الدراسة على عشرين فأراً من الفئران البيضاء وتصنيفها إلى أربع مجموعات (ن = ٥) أبتت المجموعة الأولى كمجموعة ضابطة الكنترول السالب ، في حين المجموعات الثلاث الأخر يتم حقنها لمدة يومين بجرعة من فينيل الهيدرازين ٤٠ ملجم / كجم / يوميا ، ثم تم تقسيمها الي ثلاث مجموعات المجموعة الضابطة الكنترول (غير المعالجة)، ومجموعتين تم علاجهما بعصير البنجر الأحمر بنسبة ٢ و ٥ مليجرام علي التوالي بواسطة الأنثوية المعدية يوميا. واستمرت الدراسة لمدة أربعة أسابيع.

وأظهرت النتائج أن البنجر الأحمر يحتوي على نسبة عالية من الفسفور ٨٧,٨ والبوتاسيوم ٦٨١,٣ والمغنيسيوم ٥٧,٩ والكالسيوم ٤٤,٧ ملجم / ١٠٠ جم. وبالإضافة إلى ذلك، فإنه يحتوي على الحديد ٢,١٢ والسيلينيوم ١,٦٦ ملجم / ١٠٠ جرام في الوزن الجاف. سجلت النتائج أن إجمالي الفينولات ٨٧٩,٣ و الفلافونيد ٢٢٣,٤ ملجم / ١٠٠ جم على الوزن الجاف. أظهرت النتائج أن إجمالي البيتاين ٣٥٢,٨ ملجم / ١٠٠ جم على الوزن الرطب.

أوضحت النتائج انخفاض رقم البيروكسيد في الدونات الذي تم معالجته ب ٥ مليجرام من مستخلص البيتاين حيث سجلت النتائج ٤,١٨ مل مكافئ / كجم في حين ان الدونات الذي تم معالجته ب ٢ مليجرام من مستخلص البيتاين حيث سجلت النتائج ٥,٢٥ مل مكافئ / كجم، مقارنة بالكنترول الموجب ٧,٨٧ مل مكافئ / كجم خلال فترة التخزين ٤ أسابيع .

وحققت النتائج زيادة كبيرة في هيموجلوبين الدم في مجموعات الفئران التي تم علاجها بعصير البنجر الأحمر ٢ ملجم ، ٥ ملجم حيث كانت ١٠,٩ و ١١,٧ على التوالي عند المقارنة بالكنترول الموجب ٧,٧٧ جم/ديسيلتر كما أظهرت النتائج وجود زيادة كبيرة في عدد كرات الدم الحمراء لوحظ من مجموعة الفئران التي تناولت ٢ ملجم و ٥ ملجم من البنجر الأحمر ١,١+٤,٧ و ٠,٩+٤,٩ على التوالي بالمقارنة بالكنترول الموجب ٠,٢+٢,٥٨ . وأظهرت النتائج وجود انخفاض كبير في الدهون الثلاثية ، والكوليسترول الكلي و LDL-C، ومستويات VLDL-C ، وزيادة كبيرة في مستوى HDL-C لوحظت في الفئران التي تناولت عصير البنجر الأحمر مقارنة بالكنترول الموجب .

توصي الدراسة:

هناك حاجة إلى المزيد الاعمال البحثية من أجل فهم أفضل الآليات للاستفادة من جذور البنجر الأحمر في مجال إعداد الأطعمة والصناعات الغذائية. حيث انه مصدرا جيدا للملونات الطبيعية، والمضافات الغذائية ومضادات الأكسدة الطبيعية وايضا له دور فعال في علاج الانيميا وخفض دهون الدم .

الكلمات المفتاحية:

البيتاين ، النشاط المضاد للأكسدة، الفينولات الكلييه ، الهيموجلوبين ، المالونالدهيد .