Quality Evaluation of Cookies Prepared from Garden Cress Seeds and Golden berry Fruits and Its Effect on Iron Deficiency Anemia in Rats

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Abstract

This research was designed to investigate the effect of cookies produced from whole garden cress seeds (WGCS) and golden berry fruits (GBF) on chemical, sensory and antioxidant properties. Effect of feeding with cookie samples on blood picture, biochemical parameters, iron in organs and bone marrow of rats with Fe-deficiency anemia was also assessed. Cookies were prepared by substituting wheat flour by 10%GBF and 5 or 10%WGCS. Results revealed that WGCS contained high values of iron, protein, ash and fiber. While GBF had significant levels of vitamin C, phenolics, flavonoids and antioxidant activity. Cookies containing 10% WGCS and 10% GBF (F4) contained the highest levels of iron (8.1mg/100g), vitamin C (30.71mg/100g), protein (9.27%) and antioxidant activity (23.80%) and this formula was the most acceptable by panelists comparing with all other cookies. The iron value in F4 was 50% higher than control, and vitamin C level was 23times more than control. Biological results showed that a significant improvement (p < 0.05) in values of hemoglobin, iron, ferritin, protein and glucose in serum and iron in spleen and liver tissues of anemic rats fed on (F4) comparing to positive group. Also, feeding anemic rats on (F4) clarified a remarkable improvement in bone marrow comparing to anemic rats. It could be concluded that cookies prepared using WGCS and GBF might help for the prevention and treatment of anemia, also, could be beneficial for people with malnutrition and diabetes. This study recommends using WGCS with GBF on a commercial scale in food products preparation to combat anemia in Egypt.

Key words: Iron deficiency anemia, *Lepidium sativum* seeds, *Physalis peruviana* fruits, cookies and antioxidant activity.

Introduction

Anemia is defined as a low concentration of hemoglobin (Hb) in the blood. It is a main and global public health problem and can cause serious complications such as weakened cognitive ability and intellectual performance, impaired immune system, decreased working capacity, higher mortality rate in pregnant women and low childbirth weight (Allen, 2000). The most popular reasons of anemia include nutritional deficiencies, especially iron deficiency, although deficiencies in folate, vitamins B12 and A are also important causes. Iron is an essential nutrient required for hemoglobin, thus production of red blood cells; it is a major portion of the hemoglobin molecule. Anemia affects almost one third of the world's population. In the worldwide, iron deficiency contributes to about 50% of all anemia cases in women and 42% of cases in children aged under 5 years (WHO, 2017). In developing countries, iron deficiency anemia (IDA) is a general health problem, particularly in Egypt (Soliman et al., 2007). According to EDHS, in Egypt, more than one in four children suffers from some degree of anemia for children under age five years. Rural children are more vulnerable to anemia than urban children (29 and 23%, respectively). In never-married girls and boys age 5-19 years, girls are more vulnerable to anemia than boys (21 and 18%, respectively). Almost 25% of ever-married women (15-49 years) are anemic women. The anemia percent is higher among women who live in rural upper Egypt (31%) than urban (20%) (EDHS, 2015).

Garden cress is an edible herb, belongs to *Brassicaceae* family and its scientific name is *Lepidium sativum*. It is native to Egypt and West Asia. Garden cress seeds (GCS) are called "Hab Al-Rashad" in Egypt (Hassan and Abdelrahman, 2019). GCS are among the most essential functional foods because of its nutritional and functional characteristics (Singh *et al.*, 2015). GCS considered as excellent grain as these are loaded with high contents of nutrients (Josephine *et al.*, 2020). GCS are important sources of iron, calcium, folic acid, vitamins C and A (Lahiri and Rani, 2020). Furthermore, GCS are excellent sources of omega-3 fatty acids, protein and fiber (Doke and Guha, 2014). Also, GCS are rich in health promoting phytochemical ingredients. Phenolic 954

compounds are among the most powerful natural antioxidants, which are answerable for a strong antioxidant activity (Kasabe *et al.*, 2012). GCS are utilized as antidiabetic (Mohamed and Hussein, 2017), hypocholesterolemic, (Althnaian, 2014), antimicrobial (Omer *et al.*, 2020) and anticancer (Mahassni and Al-Reemi, 2013). Furthermore, GCS are used in lactating mother's diet to increase milk secretion through the postpartum period, also, aid to regulate menstruation and thyroid hormone concentration (Chaudhary and Gupta, 2017 and Sciarrillo *et al.*, 2018). In addition, GCS are suitable for treating of hepatotoxicity (Zamzami *et al.*, 2019). Moreover, GCS are utilized to improve vitamin C deficiency, strengthen the immune system (Fleming, 1998 and Mahassni and Khudauardi, 2017) and bone healing (Abdallah *et al.*, 2020).

Physalis peruviana known as golden berry or cape gooseberry, belongs to the family Solanaceae and are grown in Egypt and other countries such as Colombia, India, South Africa, New Zealand and Kenya (Hassanien, 2011). Golden berry fruits (GBF) also named as harankash in Egypt. GBF are highly nutritious, containing high values of carotenoids, vitamin C, vitamin B-complex and minerals (Ramadan and Morsel, 2003). GBF contain iron, phosphorus and potassium. Iron value in GBF are approximately 1.47 mg/100g which is 5 to 15 times higher comparing with strawberry, apple, orange and beans (Rodrigues et al., 2009). GBF have the therapeutic property. Where GBF are utilized for treating diabetes (Sathyadevi et al., 2014) and hepatotoxicity (Al-Olayan et al., 2014). Also, GBF prevent liver inflammation as well as insulin resistance induced through obesity (Fuente et al., 2020). Furthermore, GBF are utilized as antimicrobial (Kamau et al., 2017) and anticancer (Badr and Naeem, 2019).

To overcome the problems caused by under-nutrition and anemia, some food-based methods are needed. GCS may be a boon for people suffering from malnutrition and anemia. GCS are a rich source of nonheme iron (iron found in hemoglobin) which is an easily absorbed dietary iron (Monsen, 1988). Also, GCS have high nutritional value, therapeutic properties, low cost and locally available, allowing people from all segments of society to include GCS in the diet and increase the nutritional value of their meals without increasing the cost of their diet. GBF have a high economic value and demand is increasing in the worldwide as fruits that have a high nutritional value and medicinal properties. GBF are available in Egypt at a cheap price and are a rich source of vitamin C, which helps iron absorption (**Ozturk** *et al.*, **2017**). Therefore, this research was intended to study quality evaluation of cookies prepared using garden cress seeds and golden berry fruits and its effect on iron deficiency anemia in rats.

Materials and Methods

Materials

GCS were purchased from an herbal shop in Sharkia Governorate, Egypt. Ripe GBF were obtained from local growers in Sharkia Governorate, Egypt. Wheat flour (72%), sugar, fat, baking powder and salt were purchased from the local market in Sharkia Governorate, Egypt. Minerals, vitamins, casein, cellulose, were purchased from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical Instruments, Sharkia Governorate, Egypt. Purchasing of gallic acid, Folin-Ciocalteu reagent and 2,2diphenyl- 1- picrylhydrazyl, were from Sigma Chemical Co. (St. Louis, MO, USA). Kits for blood analysis were obtained from Alkan-Medical Division Biocon, Germany.

Methods

Preparation of raw materials

WGCS were roasted at 150°C for 3-5 min until a notable aroma of seeds comes (Jain and Grover, 2017). After cooling, WGCS were ground in laboratory milling. Packing of roasted seeds flour were in air-tight plastic bags and stored in an air sealed container till use. GBF were de-husked, washed and mixed in a blender to get a smooth puree.

Preparation of Cookies

Cookies were made as described by **Erben** *et al.*, (2014). The base formula was contained: wheat flour (100g), sugar (25g), fat (25g), salt (0.8g), baking powder (1g) and water (as required). Four kinds of cookies were prepared. Control cookies (F1) were prepared as follows: solid materials were mixed, then water was 956 added and mixed for 6 min. After mixing, the dough was rolled, then it was allowed to rest for 1 min. The dough was cut with a dough cutter of 40 mm diameter. Cookies were baked at 160°C for 15 min, then allowed to cool for one hr. Cookies were packaged in polyethylene bags and stored in air-tight containers till used. While, in the other formulas, wheat flour was substituted by fresh GBF (equal to 10% as dry weight), where 10% wheat flour replaced with 53.6 g fresh GBF (moisture content 81.34%) and this formula served as (F2), whereas, in formulas F3 and F4, wheat flour was replaced with 10% GBF, in addition, 5 or 10% WGCS, respectively.

Determination of chemical composition and nutritional value

Raw materials and cookie samples were examined for moisture, protein, fiber, fat and ash according to **AOAC**, (2005). Calculation of total carbohydrates were by difference. Calculation of energy value (EV) was done by utilizing the Atwater factors (9, 4 and 4 kcal/g) of fat, protein and carbohydrate, respectively (Chaney, 2006). Vitamin C values of raw materials and cookies were estimated according to **Tandon and Pal**, (2017).

DPPH assay

The free radical scavenging activity of the ethanol extracts for raw materials and cookies were measured by 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay as described by **Brand-Williams** *et al.*, (1995).

Total phenolic content determination (TPC)

TPC was estimated by the Folin–Ciocalteu method according to **Singleton** *et al.*, (1999). TPC was expressed as mg of gallic acid equivalents (GAE) per 100 g of sample.

Total flavonoids content determination (TFC)

TFC was measured using the colorimetric assay according to **Zhishen** *et al.*, (1999). TFC was expressed as mg of quercetin equivalents (QE) per 100 g of sample.

Sensory properties of cookies

Cookie samples were evaluated for its sensory attributes after baking by twenty semi trained panelists from the academic staff members of the Dept. of Food Sci., Fac. of Agri., Zagazig Univ., Egypt. They were asked to score the properties of cookie samples including color, taste, odor, crispness, appearance and overall acceptability using the evaluate sheet according to **Saleh** *et al.*, (2012).

Biological evaluation Experimental design

Thirty-six healthy weanling male Wistar albino rats weighting 77 ± 5 g (aged 3 weeks) were utilized in the current study. Rats were kept separately in stainless steel cages in a controlled environment at $24\pm2^{\circ}$ C, relative humidity 40-60% and 12 hours light and dark cycle. Rats were maintained with free access to water and diet *ad libitum* during the test period. Rats were consumed standard diet, prepared according to **Reeves** *et al.*, (1993), for seven days as adaptation period.

For induction of IDA, rats were consumed an AIN-93M diet deficient in iron for four weeks (**Reguła** *et al.*, **2016**). Hemoglobin was determined weekly, and rats were considered as anemic, if Hb values of rats were 8-9 g/dl in current study.

Rats were separated into six groups (n=6) as follows: Group 1(G1) served as normal rats, consumed standard diet and considered as a negative control group. While, the other rat groups were anemic rats consumed Fe-deficient diet mixed with cookies, where the ratio of starch in the standard diet to cookies was 1:3 in treated rats, whereas positive control group (G2) was maintained on Fe-deficiency diet only. Groups (G3), (G4), (G5) and (G6) anemic rats consumed Fe- deficient diet containing cookies F1, F2, F3 and F4, respectively, as the previously mentioned ratio between starch and cookies.

Food intake was recorded daily throughout the test period. The body weight of rats was also recorded once a week. Treatments continued for ninety days. At the end of the test period, animals were fasted overnight, and the blood was collected in two parts. The first part of the blood was withdrawn from retroorbital venous plexus puncture under mild diethyl ether anesthesia with heparinized capillary tubes and blood samples were collected into EDTA tubes (1mg/ml), then mixed thoroughly to prevent clotting. The second part of the blood was collected via cardiac puncture into tubes without anticoagulant and centrifuged at 3000 rpm for 20 min to obtain the serum and stored at -20° C until further analyses. Thereafter, rats were sacrificed. Spleen and liver were separated, rinsed with a physiological saline solution, blotted on filter paper and immediately stored at -20° C until determine the iron.

Blood picture analysis

Hemoglobin (Hb), hematocrit (HCT), red blood cell count (RBC), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and mean cell volume (MCV) were assessed using **Dacie and Lewis**, (1984) methods.

Determination of iron, ferritin, total iron bending capacity (TIBC) and transferrin saturation (TS) levels

Serum iron was estimated according to **Dreux**, (1977) method. TIBC were estimated by **Piccardi** *et al.*, (1972) method. Serum ferritin was assessed using **Flowers** *et al.*, (1986) method. Transferrin saturation percentage was calculated as a result of the formula of serum iron/TIBC x 100.

Determination of iron level in spleen and liver tissues

The total amounts of Fe in digested samples (liver and spleen) were estimated by atomic absorption spectrophotometry (Buck scientific model 210 VGP, USA) as described by **Artimon** *et al.*, (2009).

Determination of glucose, protein and albumin in serum

Glucose was determined using **Trinder**, (1969) method. Total protein was measured as described by **Doumas**, (1975). Measuring level of albumin was done using **Doumas** *et al.*, (1971) method.

Bone marrow assessment

Bone marrow of animals were neatly separated from bone femur and examined by **Harvey**, (1984) method.

Statistical analysis of data

Data were examined statistically using the statistical software program SPSS, ver. 25. All values are expressed as Mean \pm SD. Statistical significance of the difference was analyzed using one way-ANOVA and post-hoc Duncan test for multiple group comparison. P-values were recognized statistically significant at p < 0.05 (Bailey, 1995).

Results and Discussion

Proximate chemical composition of WGCS, GBF and wheat flour (WF)

Chemical composition indicated that significant differences (p < 0.05) between WF, GBF and WGCS (Table1). Current data clarified that WGCS had the highest levels of protein (23.52%). fat (25.07%), fiber (10.50%) and ash (4.53%) comparing to WF and GBF. While GBF contained the highest value of moisture (81.34%) followed by WF (12.61%) and WGCS (3.66%). In addition, WF contained the highest carbohydrate level (75.11%) followed by WGCS (32.72%) and GBF (9.66%). Salama et al., (2019) reported that WGCS had 23.12% protein and 11.99% fiber. Chemical composition of GBF showed that protein, fat, fiber and ash were 1.95, 0.80, 5.41 and 0.84%, respectively. Chemical composition of GBF estimated during this study is in concurrence with obtained results of Rodrigues et al., (2009) for protein & ash and Briones-Labarca et al., (2013) for fiber. Also, results exhibited that the nutrient amount of WF was protein (10.50%), fat (1.04%), fiber (0.18%) & ash (0.56%), and these findings are in concurrence with Dewidar and El-Kherbawy, (2019).

Ingredient %	Raw Material					
	Wheat flour	Golden berry fruits Whole garden cress				
Moisture	12.61±0.20 ^b	81.34±1.00 ^a	3.66±0.10 ^c			
Protein	10.50±0.10 ^b	1.95±0.04 ^c	23.52±0.20 ^a			
Fat	1.04 ±0.02 ^b	0.80 ± 0.01^{b}	25.07±0.30 ^a			
Fiber	0.18±0.01 ^c	5.41±0.10 ^b	10.50±0.20 ^a			
Ash	0.56±0.01 ^c	0.84±0.01 ^b 4.53±0.10 ^a				
Carbohydrate	75.11±0.90 ^a	9.66±0.20 ^c	32.72±0.30 ^b			

 Table (1): Proximate chemical composition of WGCS, GBF and WF (on wet weight basis).

Values within the same row have different superscript letters are significantly different at (P < 0.05).

Results given in Fig. (1-3) show that TPC in GBF was 137.30 mg GAE/100g on wet weight basis (735.8 mg GAE/100g as dry weight) and this result is in concurrence with the finding reported by Yıldız et al., (2015). Furthermore, TFC in GBF was 18.36 mg QE/100g on wet weight basis (98.40 mg QE/100g as dry weight) and this finding is close to that estimated by López et al., (2013) (99.25 mg OE/100g as dry weight). Meanwhile, the antioxidant activity was 77.2% in GBF. The current result is close to the finding found by Aamer, (2018) who indicated that golden berry had 76.83% antioxidant activity. Whereas, WGCS had 357.9 mg GAE/100g TPC on wet weight basis (371.5 mg GAE/100g as dry weight) and 50.20 mg QE/100g TFC on wet weight basis (52.1 mg QE/100g as dry weight) and these findings are in concurrence with those found by Abdel-Aty et al., (2019). Moreover, WGCS contained 71% antioxidant activity, and this finding is in concurrence with the data published by Aydemir and Seda, (2011).

Results presented in Fig. (4) illustrated that GBF contained a high value of vitamin C. Vitamin C value of GBF was 42.70 mg/100g on wet weight basis (228.83 mg/100g as dry weight), which is very close to the result found by **Ramadan and Morsel**, (2004) (43.00 mg/100g on wet weight basis). Furthermore, there was 69.00 mg/100g vitamin C in roasted WGCS (71.62 mg/100g as dry weight). **Rajshri and Haripriya**, (2018) stated that vitamin C value was 77.22 mg/100g in roasted GCS. As given in Fig. (5) the iron content was recorded 27.84 mg/100g in WGCS (28.90 mg/100g as dry weight) and this data is close to the result obtained by **Halaby** *et al.*, (2015). While data showed that GBF contained 1.44 mg/100g iron on wet weight basis (7.72 mg/100g as dry weight) and this result agree with **Rodrigues** *et al.*, (2009).



Proximate chemical composition and nutritional value of cookie samples

Chemical composition of cookie samples is summarized in Table (2). Results exhibited that control cookies recorded low contents of moisture (2.40%), fiber (0.14%), ash (0.40%), vitamin C (1.30 mg/100g), iron (5.40 mg/100g), TPC (10.00 mg GAE/100g), TFC (2.33 mg QE/100g) and antioxidant activity (10.69%) comparing with all other treated cookie samples. Meanwhile, moisture (2.92%), fiber (2.01%), ash (0.81%), vitamin C (23.81 mg/100g), iron (5.66%), TPC (82.20 mg GAE/100g), TFC (11.90 mg QE/100g) and antioxidant activity (17.34%) levels significantly elevated (P < 0.05) comparing with control in cookies containing 10% GBF. In addition, all ingredient contents except carbohydrate and energy values significantly

increased (P < 0.05) with increasing the amount of WGCS flour added to cookie samples. The highest moisture value (3.44%) was recorded in formula (F4), whereas the lowest amount (2.40%) was in control cookies (F1). Findings indicated that moisture content significantly increased (P < 0.05) in cookies containing 10% GBF comparing to control cookies. Also, increasing the amount of WGCS in cookies resulted to a significant elevate (P < 0.05) in moisture level of cookies compared to cookies containing 10% GBF or control cookies. These results probably are because of the increase in water retention ability of fibers.

Results clarified that cookies containing 10% GBF and 10% WGCS (F4) contained the highest levels of protein, ash and fiber comparing with cookies produced from 10% GBF or control. These results might be due to that WGCS contain the highest levels of protein, fiber and ash which reach to 23.52, 10.50 and 4.53%, respectively, comparing with WF or GBF. The protein value of cookies (F4) increased by 22.13% comparing to F2. While there was no significant difference observed in protein level of cookies containing 10% GBF and control. Moreover, the ash and fiber contents in F4 were approximately 3-fold and 21-fold, respectively, more than control cookies.

Fat level significantly decreased (p < 0.05) in cookies produced from 10% GBF comparing with control. This result perhaps is because of GBF contain the lowest amount of fat (0.80%) compared to WF and WGCS. Fat level in F4 significantly increased (P < 0.05) comparing to F2. This finding probably is due to WGCS contain the highest content of fat (25.07%) comparing with GBF (0.8%) and WF (1.04%). Meanwhile, results did not record a significant difference between F4 and control cookies in fat content.

The level of carbohydrate significantly decreased (p < 0.05) with increasing the amount of WGCS added to the cookie samples comparing with all other cookies. These results probably are because of these cookies have higher contents of protein, fiber, ash and moisture comparing with control and GBF cookies, which resulted to a reduce in carbohydrate level. Also, energy values significantly decreased (p < 0.05) in cookies (F2-F3-F4) comparing to control cookies. These findings agree with those reported by

Shalaby, *et al.*, (2016) who observed that adding *Physalis pubescens* paste to rusk led to elevate in contents of moisture, ash and fiber, whereas, carbohydrate value decreased comparing with control. These data agree with **Dewidar and El-Kherbawy**, (2019) who stated that moisture, protein, fat, ash and fiber values increased, while, carbohydrate value decreased when GCS added to brioche comparing to control.

Regarding vitamin C level of cookie samples, results clarified that vitamin C value significantly increased (P < 0.05) in cookies produced from 10% GBF and the increase was approximately 18-times compared with control cookies. This result perhaps is due to GBF contain the highest value of vitamin C (228.83 mg/100g as dry weight) comparing to WGCS and WF. Also, WGCS contained level of vitamin C (71.62 mg/100g as dry weight) resulted to elevate the amount of vitamin C in cookies made from both 10% GBF and different level of WGCS more than cookies produced from 10% GBF only or control cookies. Data estimated that the highest value of vitamin C was in F4 and this formula contains almost 23-times vitamin C more than control cookies. **Embaby and Mokhtar, (2019)** reported that nectar containing golden berry juice contained higher level of vitamin C than control.

Statistical analysis demonstrated that the iron content was significantly increased (P < 0.05) in cookies made using WGCS at different level comparing to control. This finding probably is due to the highest content of iron in WGCS compared to GBF and WF. Meanwhile, the iron content significantly increased (P < 0.05) in cookies prepared using 10% GBF compared with control. This result may be due to that GBF have iron content more than WF. The highest iron content found in F4 (8.1 mg/100g) and the iron value in this formula was 50% higher than control cookies. **Rana and Kaur, (2016)** estimated a significant elevate in the iron amount of GCS biscuits compared with control.

Antioxidant activity, TPC and TFC significantly increased (P < 0.05) in F2, F3 and F4 comparing to control cookies. These results perhaps are attributed to increase the value of antioxidant activity, TPC and TFC in GBF and WGCS comparing to WF. F4 contained almost 11-times TPC, 7-times TFC and 2-times

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antioxidant activity more than control cookies. **Aamer**, (2018) reported that jam prepared with 35% cape gooseberry contained 24.76% antioxidant activity. **Alshehry**, (2019) noticed that biscuits containing various values of GCS (2.5-10%) significantly elevated in antioxidant activity, TPC and TFC.

	Cookie sample					
Ingredient	F 1	F2	F3	F4		
Moisture%	2.40 ± 0.11^d	$2.92 \pm 0.12^{\circ}$	3.20 ± 0.12^b	3.44 ± 0.13^{a}		
Protein %	7.66 ± 0.16^{c}	7.59 ± 0.14^{c}	8.40 ± 0.15^{b}	9.27 ± 0.16^a		
Fat%	16.40 ± 0.22^{a}	$15.20 \pm 0.21^{\circ}$	15.60 ± 0.23^{bc}	16.05 ± 0.21^{ab}		
Fiber%	0.14 ± 0.01^d	$2.01 \pm 0.01^{\circ}$	2.53 ± 0.02^{b}	2.99 ± 0.03^a		
Ash%	0.40 ± 0.01^{d}	$0.81 \pm 0.02^{\circ}$	1.03 ± 0.02^{b}	1.30 ± 0.03^a		
Carbohydrate%	73.00 ± 1.15^{a}	71.47 ±1.12 ^a	69.24 ±1.10 ^b	66.95 ±1.10 ^c		
*EV (Kcal/100g)	470.24 ± 2.21^{a}	453.04 ± 2.11^{b}	$450.96 \pm 2.22^{\circ}$	449.33 ± 2.12^{d}		
Vitamin C (mg/100g)	1.30 ± 0.01^d	$23.81 \pm 0.10^{\circ}$	26.83 ± 0.12^{b}	30.71 ± 0.18^a		
Iron (mg/100g)	5.40 ± 0.10^{d}	$5.66 \pm 0.12^{\circ}$	6.85 ± 0.20^{b}	8.10 ±0.24 ^a		
TPC (mg GAE/100g)	10.00 ± 0.33^{d}	$82.20 \pm 1.10^{\circ}$	100.00 ± 1.44^{b}	116.50 ± 1.53^{a}		
TFC (mg QE/100g)	2.33 ± 0.11^{d}	$11.90 \pm 0.13^{\circ}$	14.22 ± 0.15^{b}	16.20 ± 0.16^{a}		
DPPH %	10.69 ± 0.20^{d}	17.34 ± 0.22^{c}	20.36 ± 0.30^b	23.80 ± 0.40^a		

Table (2): Proximate chemical composition and nutritional value of cookie samples (on wet weight basis).

Values within the same row have different superscript letters are significantly different *at (P< 0.05)*. *EV: Energy value.F1: Control cookies.F2: Cookies containing 10%GBF.F3: Cookies containing 10%GBF + 5%WGCS.F4: Cookies containing 10%GBF + 10%WGCS.

Sensory evaluation of cookies

The web diagram for mean sensory evaluation scores of cookie samples demonstrated that mean scores of color, taste, odor, crispness, appearance as well as overall acceptability of control cookies (F1) were 8.60, 8.80, 8.60, 9.00, 8.70 and 8.80, respectively, of total scores (10) (Fig. 6). Whereas, the mean scores of same attributes for F2, F3 and F4 significantly increased comparing with control cookies. Data clarified that cookies prepared with 10% WGCS and 10% GBF (F4) recorded the highest scores for all attributes tested comparing to control. The mean scores of same parameters for the best cookies (F4) were 9.50 (color), 9.52 (taste), 9.25 (odor), 9.20 (crispness), 9.44 (appearance) and 9.50 (overall acceptability). **Shalaby**, *et al.*, 965

(2016) reported that there was no significant difference between rusk containing 10% golden berry and control rusk. Rana and Kaur, (2016) stated that biscuits containing 10% GCS recorded the highest scores in all sensory attributes comparing with control.



Fig. (6): Web diagram for mean sensory evaluation scores of cookies.

Biological evaluation

Data in Table (3) clarified that a significant decrease (p < 0.05) in BWG, FI and FER values of rats fed on Fe-deficient diet (G2), which recorded 66.00g, 945g and 0.07, respectively, comparing with control group, accordingly, iron is important for the growth of rats, as reported by **Strube** *et al.*, (2002). These findings probably are due to rats fed on Fe-deficient diet have lower plasma thyroid hormone values than the healthy control group (Beard *et al.*, 1998). Similar findings were published by **Thakur** *et al.*, (2019) who noticed that BWG and FI values were markedly lower in rats fed with Fe-deficient diet comparing to control rats. Results exhibited that BWG, FI and FER values of anemic rats consumed cookies containing 10% GBF significantly increased (p < 0.05) comparing with positive control rats or anemic rats consumed cookies and these data are in agreement with the observation of Badr and Naeem, (2019). Moreover, data

clarified that a significant increase (P < 0.05) in BWG, FI and FER values of anemic rats consumed cookies prepared with 5% WGCS + 10% GBF or 10% WGCS + 10% GBF comparing to anemic rats or anemic rats fed on other cookies, and these data are in concurrence with the results published by **Balgoon**, (2019).

Group	Initial body weight (IBW) (g)	Final body weight (FBW) (g)	Body weight gain (BWG) (g)	Food intake (FI) (g/rat/90 days)	Feed efficiency ratio (FER)	
G1	77.00 ± 0.60^{e}	197.00 ± 1.50^{b}	120.00 ± 1.00^{a}	1314 ± 2.30^a	0.091 ± 0.003^a	
G2	81.50 ± 0.65^a	$147.50\pm1.30^{\rm f}$	$66.00\pm0.50^{\rm f}$	$945\pm1.50^{\rm f}$	0.070 ± 0.001^{e}	
G3	$79.91 \pm 0.70^{\circ}$	148.62 ± 1.31^{e}	68.71 ± 0.71^{e}	972 ± 1.60^{e}	0.071 ± 0.001^{d}	
G4	78.82 ± 0.71^d	157.05 ± 1.54^{d}	78.23 ± 0.77^d	1062 ± 1.80^{d}	0.074 ± 0.001^{c}	
G5	80.66 ± 0.70^{b}	$177.46 \pm 1.50^{\circ}$	$96.80 \pm 0.66^{\circ}$	1170 ± 1.85^{c}	0.083 ± 0.002^b	
G6	82.00 ± 0.81^{a}	199.00 ± 1.61^{a}	117.00 ± 0.80^{b}	1305 ± 2.10^{b}	0.090 ± 0.002^{a}	

 Table (3): Effect of feeding anemic rats with cookies containing WGCS

 and GBF on FBW, BWG, FI and FER.

Values within the same column have different superscript letters are significantly different at (P < 0.05). G1: Normal group. G2: Positive control group. G3: Anemic rats fed on control cookies. G4: Anemic rats fed on 10%GBF cookies. G5: Anemic rats fed on 10%GBF + 5%WGCS cookies. G6: Anemic rats fed on 10%GBF + 10%WGCS cookies.

Data in Table (4) exhibited that hematological parameters values significantly decreased (p < 0.05) in anemic rats consumed Fe-deficient diet (G2) comparing with control rats. These findings agree with data estimated by Thakur et al., (2019) who found that rats fed on Fe-deficient diet for 35 days indicated a significant decrease (p<0.05) in Hb, RBC, HCT, MCV, MCH and MCHC values comparing with normal control rats. A reduce in hemoglobin level probably is due to iron, which has a large role in hemoglobin production. Results clarified that anemic rats consumed diets containing control cookies or 10% GBF cookies did not show significant differences (p < 0.05) in hematological parameters values compared to anemic rats. Anemic rats fed on diets containing cookies prepared with WGCS at various levels significantly elevated (p < 0.05) in caused hematological parameters values compared with anemic rats. When the amount of WGCS in cookies increased, Hb, HCT, RBC, MCV, MCH and MCHC levels were also increased. These results probably are due to WGCS contained the highest value of iron compared to GBF and WF. Feeding anemic rats with cookies prepared using 10%

WGCS + 10% GBF revealed the highest contents of hematological parameters, which were close to the normal values of control rats. **Verma**, (2019) stated that significantly elevated in Hb and PCV levels of chick treated with a different level of GCS comparing to control.

 Table (4): Effect of feeding anemic rats with cookies containing WGCS

 and GBF on hematological parameters values.

Group	Hematological parameter						
	Hb (g/dl)	HCT (%)	RBC (Million/mm ³)	MCV (fL)	MCH (pg)	MCHC (g/dl)	
G1	14.00 ± 0.20^a	45.60 ± 0.40^a	4.40 ± 0.15^{a}	85.60 ± 1.10^{a}	29.80 ±0.32 ^a	35.90 ±0.45 ^a	
G2	$8.30\pm0.12^{\rm c}$	30.50 ± 0.22^{d}	2.90 ± 0.10^{c}	$72.00\pm1.00^{\rm c}$	25.00 ± 0.20^{c}	$30.00 \pm 0.32^{\circ}$	
G3	8.40 ± 0.11^{c}	30.60 ± 0.21^{d}	$3.05 \pm 0.11^{\circ}$	$72.20 \pm 1.10^{\circ}$	25.20 ± 0.21^{c}	$30.10 \pm 0.31^{\circ}$	
G4	8.93 ± 0.13^{c}	31.13 ± 0.34^d	$3.20\pm0.12^{\rm c}$	72.80±1.11 ^c	$25.76\pm0.27^{\text{c}}$	30.70 ± 0.37^{c}	
G5	11.61 ± 0.14^{b}	$37.32 \pm 0.35^{\circ}$	3.84 ± 0.13^b	79.65 ± 1.22^{b}	27.31 ± 0.26^{b}	33.16 ± 0.36^b	
G6	13.84 ± 0.20^a	44.10 ± 0.41^{b}	4.38 ± 0.14^{a}	84.81 ± 1.31^{a}	29.11 ± 0.30^a	35.00 ± 0.40^a	

Values within the same column have different superscript letters are significantly different at (*P* < 0.05). G1: Normal group. G2: Positive control group. G3: Anemic rats fed on control cookies. G4: Anemic rats fed on 10%GBF cookies. G5: Anemic rats fed on 10%GBF + 5%WGCS cookies. G6: Anemic rats fed on 10%GBF + 10%WGCS cookies.

Iron, ferritin, TIBC and TS values of anemic rats fed on different cookie diets are illustrated in Table (5). Resulted showed that iron, ferritin and TS contents significantly decreased (p < 0.05) in anemic rats fed with Fe-deficient diet (G2) comparing with the control group. The level of Fe-deficiency produced by Fedeficient diet was severe sufficient to reduce FBW and BWG, as previously stated in the result of present study. Current results are in concurrence with Thakur et al., (2019) who observed that iron, ferritin, and TS levels significantly decreased in rats consumed Fe-deficient diet than control rats. Findings indicated that anemic rats fed on control cookies or 10% GBF cookies did not register significantly elevated (p < 0.05) in iron values comparing with anemic rats. Feeding anemic rats with 10% GBF + 5 or 10% WGCS cookie diets exhibited significantly higher (P < 0.05) values of iron comparing with G2 or G3 or G4. These results perhaps due to that WGCS have the highest content of iron compared to WF and GBF. Furthermore, serum iron increased by 1.0% in anemic animals fed on 10% GBF cookies, while serum iron elevated by 968 =

20.3 and 32.8% for anemic rats fed on 10% GBF + (5 or 10%) WGCS cookie diets, respectively, comparing with anemic rats. The highest value of serum iron was noticed in anemic animals fed on cookies containing 10% GBF + 10% WGCS comparing to anemic rats. This value was actually close to the value of a healthy group. The data also indicated that significantly decreased (P < 0.05) in TS and ferritin values after feeding on Fe-deficient diet (G2) and these values significantly elevated (P < 0.05) after feeding on 10% GBF + 5 or 10% WGCS cookie diets (G5 and G6). The highest levels of TS and ferritin were estimated in anemic rats consumed cookies containing 10% WGCS and 10% GBF comparing to anemic rats. These values were close to values of control rats. Whereas, after feeding rats with Fe-deficient diet (G2) for 90 days, TIBC value significantly elevated (P < 0.05) comparing with normal rats due to gradual Fe depletion from the body stores. Similar result was published by Thakur et al., (2019) who found that TIBC level of rats fed on Fe-deficient diet significantly elevated comparing to control rats. Feeding anemic rats with control cookies or 10% GBF cookies did not show significant differences (P < 0.05) in TIBC levels comparing with anemic rats. While anemic rats feeding with cookie diets containing 10% GBF + 5% WGCS or 10% GBF + 10% WGCS led to a significant reduce (P < 0.05) in TIBC levels compared with anemic rats. The TIBC value of rats fed on cookies containing 10% WGCS and 10% GBF was close to the value of normal rats.

Table (5): Effect of feeding anemic rats with cookies containing WGCS and GBF on iron, ferritin, total iron bending capacity (TIBC) and transferrin saturation (TS) in serum.

Group	Biochemical parameter						
	Iron (µg/dl) Ferritin (ng/ml)		TIBC (µg/dl)	TS (%)			
G1	75.40 ± 0.64^a	25.00 ±0.20 ^a	289.00 ± 1.50^{d}	26.09 ± 0.23^a			
G2	55.20 ± 0.45^d	15.40 ± 0.10^{d}	380.50 ± 1.40^{a}	14.51 ± 0.10^{d}			
G3	55.30 ± 0.50^d	15.70 ± 0.11^{d}	380.30 ± 1.41^{a}	14.54 ± 0.11^{d}			
G4	55.70 ± 0.61^{d}	16.20 ± 0.12^{d}	379.60 ± 1.37^{a}	14.67 ± 0.11^{d}			
G5	$66.38 \pm 0.66^{\circ}$	$19.29 \pm 0.12^{\circ}$	331.58 ± 1.26^{b}	20.02 ± 0.15^{c}			
G6	73.30 ± 0.61^{b}	21.44 ± 0.13^{b}	290.44 ± 1.12^{c}	25.24 ± 0.20^{b}			

Values within the same column have different superscript letters are significantly different at (P < 0.05). G1: Normal group. G2: Positive control group. G3: Anemic rats fed on control cookies. G4: Anemic rats fed on 10%GBF cookies. G5: Anemic rats fed on 10%GBF + 5%WGCS cookies. G6: Anemic rats fed on 10%GBF + 10%WGCS cookies.

Table (6) shows protein, albumin and glucose levels of rat groups fed on test diets. Data showed that significantly decreased (P < 0.05) in albumin and protein values after feeding rats with Fedeficient diet compared with a healthy group. Current data clarified that significantly elevated (P < 0.05) in the protein level after feeding anemic rats on 10% GBF + 5 or 10% WGCS cookie diets comparing with the anemic group. The albumin value increased when WGCS at various levels were added to 10% GBF to prepare cookies in the diet of anemic rats. The maximum albumin and protein values were recorded in anemic rats consumed cookies containing 10% WGCS and 10% GBF. Sanad et al., (2018) proved that the efficiency of chloroformic and methanolic extracts of GCS in raising the protein and albumin values in contaminated rats with Aflatoxin. Also, the data in Table (6) show significant differences (P < 0.05) between anemic group and test groups in glucose levels. Comparing with negative control rats, findings demonstrated that significantly elevated (P < 0.05) in the glucose values of anemic rats, and this result is in concurrence with the finding obtained by Kamei et al., (2010). Results confirmed that significantly reduced (P < 0.05) in glucose contents of anemic rats consumed diets containing 10% GBF cookies or 10% GBF + (5 or10%) WGCS cookies comparing with anemic rats. These findings probably are result to GBF and WGCS have higher amount of phenolic and flavonoids compounds compared with WF. Shalaby, et al., (2016) stated that significantly decreased in blood sugar contents of rats with diabetes consumed rusk containing 20% Physalis pubescens L. comparing with diabetic rats. Kamani et al., (2017) indicated that GCS extract significantly decreased blood sugar levels of rats with diabetes compared with diabetic rats. The best result was in anemic rats consumed cookies containing both 10% WGCS and 10% GBF comparing with anemic rats, where the level of glucose reached to normal level.

Group	Biochemical parameter				
	Protein (g/dl)	Albumin (g/dl)	Glucose (mg/dl)		
G1	7.20 ± 0.15^a	4.40 ± 0.12^a	105.30 ± 1.00^{d}		
G2	$5.20 \pm 0.11^{\circ}$	3.20 ± 0.10^b	122.50 ± 1.20^{a}		
G3	$5.26\pm0.12^{\rm c}$	3.22 ± 0.10^{b}	122.00 ± 1.11^{a}		
G4	$5.24 \pm 0.11^{\circ}$	3.21 ± 0.11^b	114.20 ± 1.10^{b}		
G5	6.07 ± 0.13^{b}	3.55 ± 0.11^{ab}	$110.00 \pm 1.12^{\circ}$		
G6	6.20 ± 0.14^{b}	3.74 ± 0.12^{ab}	$105.50 \pm 1.10^{\rm d}$		

 Table (6): Effect of feeding anemic rats with cookies containing WGCS

 and GBF on protein, albumin and glucose in serum.

Values within the same column have different superscript letters are significantly different at (P < 0.05). G1: Normal group. G2: Positive control group. G3: Anemic rats fed on control cookies. G4: Anemic rats fed on 10%GBF cookies. G5: Anemic rats fed on 10%GBF + 5%WGCS cookies. G6: Anemic rats fed on 10%GBF + 10%WGCS cookies.

Both spleen and liver are the major organs of iron storage and play a vital role in iron metabolism (Standal et al., 1997). Data clarified in Table (7) show that iron levels of spleen and liver in rats with anemia significantly decreased (p < 0.05) comparing with normal group and these findings are in concurrence with He et al., (2019). Low iron values in spleen and liver of animals fed on Fe-deficient diet showed severe depletion of storage iron (He et al., 2019). Results exhibited that no significant improvement (p < 0.05) was estimated in rats with anemia treated with either control cookies or 10% GBF cookies comparing to anemic rats. Compared to anemic rats, iron amounts of spleen and liver significantly elevated (p < 0.05) when anemic rats fed on 10% GBF + 5 or 10% WGCS cookie diets. The highest iron values were estimated in spleen and liver (16.20 and 6.55 mg/100g tissues), respectively, in anemic rats fed on cookies containing 10% WGCS and 10% GBF. These values were actually close to a healthy group.

Group	Organs iron content (mg /100g tissues)			
	Liver	spleen		
G1	6.80 ± 0.14^a	20.50 ±0.25 ^a		
G2	$5.50 \pm 0.12^{\circ}$	14.20 ± 0.17^{d}		
G3	5.62 ± 0.11^{bc}	14.30 ± 0.18^d		
G4	5.70± 0.13 ^{bc}	14.50 ± 0.19^{d}		
G5	6.21 ± 0.16^{ab}	$15.34 \pm 0.20^{\circ}$		
G6	6.55 ± 0.13^{a}	16.20 ± 0.21^{b}		

 Table (7): Effect of feeding anemic rats with cookies containing WGCS

 and GBF on iron values of spleen and liver tissues.

Values within the same column have different superscript letters are significantly different at (P<0.05).</th>G1: Normal group.G2: Positive control group.G3: Anemic rats fed on controlcookies.G4: Anemic rats fed on 10%GBF cookies.G5: Anemic rats fed on 10%GBF +5%WGCS cookies.G6: Anemic rats fed on 10%GBF + 10%WGCS cookies.

Bone marrow assessment:

Photo. (1-6) illustrate bone marrow assessment of different experimental groups. From Photo. (1), bone marrow assessment of normal group revealed that normal cellularity of different hematopoietic series which including myelocytic, lymphocytic and erythrocytic components beside normal megakaryocytic contents. Bony spicules of spongy bone, moderate number of fat vacuole with dilated sinusoids were commonly observed. Whereas, bone marrow assessment of anemic group indicated that hypocellular less active bone marrow which represented by decrease number of myelocyte, lymphocytic, erythrocytic and megakaryocytic hematopoietic series. Moreover, higher amount of adipocyte with bony spicules were also detected (Photo. 2). These findings may be because of the severity of the iron deficiency resulting from Fe-deficient diet. These results are concurrent with Soltan, (2013). Roodenburg et al., (2000) stated that iron insufficiency affects the proportion of myeloid erythroid in bone marrow smears as well as production of the blood reticulocyte. Bone marrow of anemic rats consumed control cookies showed that hypocellularity of different hemopiotic series and presence of numerous fat cells dispersed throughout the marrow. Furthermore,

a number of megakaryocytes and bone trabeculae were also noticed (Photo. 3). In addition, bone marrow assessment of rats with anemia fed on 10% GBF cookies showed that low active bone marrow with number of megakaryocytes. Presence amount of lipocyte and bony spicules were also observed (Photo. 4). While, bone marrow for anemic rats consumed 5% WGCS + 10%GBF exhibited that active hypercellular bone marrow with increased number of all hematopoietic series with presence of a large number of mature lymphocytes and megakaryocytes. Lesser amount of adipocyte with bony spicules were also detected (Photo. 5). Finally, photomicrograph of anemic rats fed on 10% WGCS + 10% GBF demonstrated that highly active hyper cellular bone marrow which represented by increase number of all hemopiotic series with presence of increase number of mature lymphocytes and megakaryocytes. The bone marrow sinusoids were seen and mildly dilated means active bone marrow with a lesser amount of adipocyte were also detected. Hence, this group showed bone marrow close to the healthy control group of rats (Photo. 6). These results perhaps are because of 10% WGCS + 10% GBF cookies contain the highest amounts of iron and vitamin C comparing with all other cookies.



Photo. 1 (G1): Photomicrograph of bone marrow showing normal cellularity of different hematopoietic series (star) beside normal megakaryocytic contents (curved arrows) and moderate number of fat vacuole (arrow) H&E X400.



Photo. 3 (G3): Photomicrograph of bone marrow showing less amount of hematopoietic series (star) with numerous fat cells (arrow) and number of megakaryocytes (curved arrows) H&E X400.



Photo. 5 (G5): Photomicrograph of bone marrow showing highly active hematopoietic series (star), lesser amount of adipocyte (arrow) and normal megakaryocytic contents (curved arrows) H&E X400.



Photo. 2 (G2): Photomicrograph of bone marrow showing less amount of hematopoietic series (star) with a high amount of lipocyte (arrow) and low number of megakaryocytes (curved arrow), in addition to bony spicules of spongy bone (arrow head) H&E X400.



Photo. 4 (G4): Photomicrograph of bone marrow showing low content of hematopoietic series (star) with number of megakaryocytes (curved arrows) and a number of fat vacuole (arrow) H&E X400.



Photo. 6 (G6): photomicrograph of bone marrow showing increase number of different hematopoietic series (star) and megakaryocytic contents (curved arrow), beside few numbers of fat vacuole (arrow) H&E X400.

Current study exhibited that the best result for bone marrow assessment was noticed in anemic rats fed on 10% WGCS + 10% GBF cookies compared to anemic rats (Table 8). This result was actually close to a healthy group. This finding is in concurrence with data of blood picture in current study. Moreover, this data is also consistent with previous results for biochemical parameters as well as iron contents in spleen and liver tissues in the current study.

Table (8): The main histological changes for bone marrow among different experimental groups of anemic rats treated with various types of cookie.

Variable	G1	G2	G3	G4	G5	G6
Myelocyte, lymphocytic and erythrocytic components	+++	+	+	+	++	+++
Number of Megakaryocytes	+++	+	+	+	++	+++
Number of fat vacuole		+++	+++	+++	++	+
			_			

+: Mild (25-39%).++: Moderate (40-65%).+++: High (up to 65%).G1: Normal group.G2: Positive control group.G3: Anemic rats fed on control cookies.G4: Anemic rats fed on 10%GBF cookies.G5: Anemic rats fed on 10%GBF + 5%WGCScookies.G6: Anemic rats fed on 10%GBF + 10%WGCS cookies.

Conclusion

IDA is considered one of the most dangerous nutritional problems. Data of the current study proved that cookies produced from 10% WGCS and 10% GBF had the highest nutritional value comparing with other cookies, particularly, in iron and vitamin C levels. Feeding with these cookies led to a significant improve (p < 0.05) in the hematological and biochemical measurements of anemic rats. This research work is considered one of the best strategies to combat anemia through food-based strategy. Where WGCS and GBF are locally available at cheap prices, also, WGCS work well to control anemia. Accordingly, this type of cookies could be beneficial for people suffering from anemia and malnutrition. Thus, these cookies might be useful for children under age five, school children, adolescents and women, especially pregnant and lactating women with anemia.

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تقييم جودة الكوكيز المحضر من بذور حب الرشاد وثمار الحرنكش وتأثيرة على انيميا نقص الحديد في الفئران

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يهدف هذا البحث إلى دراسة تأثير الكوكيز المحضر من بذور حب الرشاد (WGCS) والحرنكش(GBF) على الخصائص الكيميائية والحسية ومضادات الأكسدة. أيضاً، تم تقييم تأثير التغذية بعينات الكوكيز على صورة الدم والقياسات الكيميائية الحيوية والحديد في الأعضاء ونخاع العظام للفئران المصابة بانيميا نقص الحديد. تم تحضير الكوكيز باستبدال دقيق القمح بنسبة 10 ٪حرنكش و5 أو 10٪ بذور حب الرشاد. وأشارت النتائج أن بذور حب الرشاد أحتوت على قيم مرتفعة من الحديد والبروتين والرماد والألياف. بينما أحتوى الحرنكش على مستويات مرتفعة من فيتامينC والمركبات الفينولية والفلافونويدات والنشاط المضاد للأكسدة. أحتوى الكوكيز المحضر من 10٪ بذور حب الرشاد و 10٪ حرنكش (F4) على أعلى مستويات من الحديد (8.1 مجم/100جم) وفيتامينC (30.71 مجم/100جم) والبروتين (9.27٪) والنشاط المضاد للأكسدة (23.80٪) وكان هذا النوع من الكوكيز الأكثر قبولًا من قبل المحكمين مقارنة بجميع أنواع الكوكيز الأخرى. وكانت قيمة الحديد في عينة الكوكيز (F4) أعلى بنسبة 50٪ من الكوكيز الكنترول، وكان مستوى فيتامينC أكثر بمقدار 23 مرة من الكوكيز الكنترول. وأظهرت النتائج البيولوجية وجود تحسناً معنوياً (p<0.05) في قيم الهيموجلوبين والحديد والفيريتين والبروتين والجلوكوز في مصل الدم والحديد في أنسجة الطحال والكبد للفئران المصابة بالانيميا التي تغذت على (F4) مقارنة بالفئران المصابة بالانيميا. كما أظهرت تغذية الفئران المصابة بالانيميا على (F4) تحسناً ملحوظاً في نخاع العظام مقارنة بالفئران المصابة بالانيميا. ويمكن أن نستنتج أن الكوكيز المحضر باستخدام بذور حب الرشاد والحرنكش قد يساعد في الوقاية من الانيميا وعلاجها، كما يمكن أن يكون مفيداً أيضاً للأشخاص الذين يُعانون من سوء التغذية ومرض السكري. وتوصى هذه الدراسة باستخدام بذور حب الرشاد مع الحرنكش على نطاق تجاري في تحضير المنتجات الغذائية لمكافحة الانيميا في مصر.

الكلمات الافتتاحية: انيميا نقص الحديد – بذور حب الرشاد – ثمار الحرنكش– الكوكيز – النشاط المضاد للاكسدة.