Evaluation of anti-obesity activity of biscuits fortified with dried red rose buds and petals on albino rats

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Abstract: The present investigation aimed to evaluate the effect of replacement wheat flour by 2.5, 5, 7.5 and 10% of dried red rose buds and petals (RBP) on the chemical composition, physical and sensory properties of biscuits and the effect of biscuits consumption on obese rats. Thirty six Albino rats were divided into six equal groups, one was kept as negative control group, while the other five groups were fed on high fat diet for month to induce obesity then one of them kept as positive control group, while the other 4 group fed on biscuits incorporated with 2.5, 5, 7.5 and 10% of RBP for 60 days by 10% of basil diet. The obtained results revealed that RBP extract contains several of phenolic and flavonoid compounds and recorded high antioxidants activity. Biscuits prepared from wheat flour containing 10% RBP had a significant hardness texture 87.73±5.7 (N) compared to control biscuits 45±1.44 (N), biscuits containing 2.5% RBP had the highest overall acceptability scores followed by 5, 7.5 and 10% but all biscuits were acceptable by panelists. Feeding rats on biscuits fortified with 7.5 and10% of RBP showed a significant reduction in triglyceride, total cholesterol, LDL-cholesterol, glucose and liver enzymes levels as well as improved of kidney functions of obese rats compared to positive control group. Also, it caused a significant *($P \leq 0.05$)* decrease in malonaldehyde (MDA) content *vs* a significant ($P \leq 0.05$) increase in anti-oxidant enzymes activities . In conclusion, data of the present investigation recommended consumption of biscuits fortified with red RBP within the daily diets of obese patients to improve serum lipid profile and loss weight.

Keywords: Dried red rose buds and petals, antioxidant activity, liver functions serum lipid profile, antioxidant enzymes**,** MDA, histopathological examination.

Introduction

Obesity is defined as an excess body fat has stored to the extent that it may have an adverse effect on human health. Causes of obesity include lifestyle, genetic factors, diet, metabolism, physical activity and the socio-cultural environmental factors (Marti *et al*., 2008). Obesity has become a very widespread global health problem. In the latest study, which analyzed data from195 countries between 1990 and 2015, stated that 603.7 million adults and 107.7 million children were obese (GBD 2015 Obesity Collaborators, 2017). It is known that obesity is occurred by increase in size or numbers of fat cells hence increase adiposity tissue mass in human body. It is related to cardiovascular and cerebrovascular diseases because it causes high of fats level in the blood which leads to high of the oxidative effort for the body, leading to high in the percent of premature mortality (National Institutes of Health, 2000). Also, it is an independent risk factor for metabolic syndrome, medical problems linked to the hypertension development, type 2 diabetes, sleep apnea, respiratory disorders, stroke and certain types of cancer (Landsberg *et al*., 2013 and Obata *et al*., 2017).

Many studies stated that the different surgeries and medical drugs for treatment of obesity have many drawbacks such as high costs and side effects. The restrict calorie in diet, natural products intake and increase physical activity are an excellent alternative ways to prevent diet-induced obesity (WHO, 2007, Mohamed *et al*., 2014 and Liu *et al*., 2017). Natural products are used as plant based dietary supplements for weight control. Also, the extracts and isolated chemical compounds from plants are used as raw material for the development of obesity treatments, so consumption of herbs is effective strategy for obesity control and weight management (Chandrasekaran *et al*., 2012)

Roses are of worldwide economic importance. They are known as an important commercial crop used as a source of essential oils for perfumes and scents. Roses belong to the family *Rosaceae* and the genus Rosa (Vun *et al*., 2013). Roses are known as edible flowers and used as raw material in the production of dried rose, rose oil, herbal tea, rose syrup, rose jam, rose water, spices, flavoring and coloring agents in the formulation of delight products and flavoring of desserts like such as ice cream, rice pudding and yogurt, owing to their brilliant color, rich aroma, and high nutritional value (Ge and Ma, 2013; Lee *et al*., 2015 and Kart and Çağınd, 2017).

In the past, roses have been used as herbal folk remedies for alleviating menstrual problems and treating blood circulation disorders. Consuming various functional beverages made with extracts from edible roses provides beneficial effects to human health (Vinokur *et al*., 2006). According to a recent study, roses have second highest antioxidant level among 30 medicinal plants. This attributed to its high content of phenolic and flavonoid compounds, resulting in high antioxidant activities (Vanderjagt *et al*., 2002). So there is a worldwide trend of using edible roses as raw material for anti-inflammatory drugs and antioxidants as well as anticancer, analgesic, sedative, anti-stress property and skin disease therapeutic (Vun *et al*., 2013 and Choi *et al*., 2015). Edible roses are also known to have efficacy for inhibiting histamine release, leading to their development as a therapeutic option for patients with allergy (Chun *et al*., 2004 and Lee *et al*., 2011). The combination of health benefits of roses and its uses in cuisine increases the possibility of using roses in functional food products. However, the available knowledge about the health-promoting potential of roses is still insufficient.

The objective of the present study was to identify and quantify phenolic compounds and evaluate antioxidant activity of dried red RBP. Also, the effect of biscuits containing RBP on obese rats will be in the scope of this investigation.

Materials and methods

Materials

Dried red RBP were purchased from the local herbal market, Zagazig City, Egypt. The dried rose was powdered using a café mill and were sieved through a 60 mesh screen and stored in a tightly sealed plastic container in the freezer at - 18° C for further uses .

Wheat flour 72% extraction, sun flower oil, corn oil, starch, salt, sugar were obtained from local market. All chemicals used in this study were bought from El-Gamhouria Company for Trading Drugs, Chemicals and Medical Instuments (Zagazig City, El-Sharkia Governorate, Egypt). Also, Casein, cellulose, vitamins and minerals ingredients while Gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin Cio-Calteau (2N) were purchased from Sigma Co. (St. Louis, MO). Thirty six adult male

albino rats (Sprague Dawley strain) were obtained from National Research Center (NRC), Dokki, Cairo - Egypt.

Formulation and Preparation of biscuits

Biscuits were prepared according to AACC, (2000) and Mesías *et al.*, (2015). The method and formula of control biscuit was 130 g wheat flour, 35g sucrose, 26 g sunflower oil, 0.8 g sodium bicarbonate, 0.4 g ammonium bicarbonate and 1 g salt. 30 g distilled water. The wheat flour was replacement by 2.5, 5, 7.5 and 10 % of rose powder. The ingredients were thoroughly mixed and the dough was rolled out to disks with the diameter of 5.6 cm and the thickness of 3 mm then baked at 190ºC for 10 min in a conventional oven (Memmert UNE 400 ،Germany). The resultant biscuits were packaged in polyethylene bags for further studies and analysis.

Determination of chemical composition

Biscuits were powdered and used for further analysis such as moisture, ash, crude fat, crude fibers and crude protein was calculated by multiplying total nitrogen value by a factor of (6.25) according to AOAC (2005) standard methods. Total carbohydrate content was estimated by difference = 100- (% crude oil + %crude protein + %ash+%moisture+ %fiber).

Extraction and fractionation of phenolic and flavonoid compounds by HPLC

Dried powder of red rose was firstly defatted by using *n*- hexan then extracted by aqueous ethanol 70% at a ratio (1:10 w/v) overnight at room temperature with shaking followed by filtration through Whatman paper (No.1) then the filtrate was evaporated in a rotary evaporator (BÜCHI-water bath-B-480, Germany) at 40°C. Extracts were freeze-dried at -58±2°C (Thermo- Electron Corporation - Heto power dry LL300 Freeze Dryer, France). The dried extracts were weighed to determine the yields and stored at -20 °C until further use. Separation and identification of phenolic and flavonoid compounds by using HPLC were carried out according to Goupy *et al.*, (1999).

Determination of total phenolics and antioxidants activity for RBP

Total phenolics were measured by using a Folin–Ciocalteu reagent as described by Zheng and Wang (2001), the phenols were measured at 765nm then the results were reported as mg of gallic acid equivalents (GAE) per 100 g of dried weight. Antioxidants activity was measured according to (Tepe *et al.*, 2005). The inhibition of free radical DPPH in percent was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = (A_c - A_s / A_c) \times 100'$$

Where: A_c is the absorbance of control reaction (containing all reagents except the test extract) and A_s is the absorbance in the presence of the tested extracts.

Physical and technological evaluation of biscuits

Spread ratio

At first, diameter of biscuits was measured by laying six biscuits edge-to-edge with the help of a scale. The same set of biscuits was rotated 90º and the diameter was re-measured which the average values of these biscuits are presented in millimeter. Second thickness of biscuits was measured by stacking six biscuits on top of one

another and taking the average of six biscuits in millimeter. The spread ratio was calculated by dividing diameter by thickness according to AACC, (2002).

Texture evaluation

Three-point bend test was used to analyze biscuits breaking force as an indicator for its hardness by using a texture analyzer TA-TX2(Stable Microsystem, Surrey, England) equipped with 25 kg load cell. The peak breaking force (N) of biscuits using the force-in-compression was recorded. Biscuits samples were placed on base beams with a distance of 5 cm between the two beams. A three – point bending rig was used with an HDP/BS, knife-edge probe. The analyzer was set at a return- to-start cycle, with a pretest speed of 2 mm/s, test speed of 2 mm/s, post-test speed of 10 mm/s, the trigger force was 20 g and distance of 20 mm **(**Abdel-Samie, *et al.*, 2010).

Color measurement

The surface color for all biscuits samples (*L**, *a** and *b**) were conducted using Hunterlab color analyzer (Hunterlab color Flex EZ, USA). Average of three values was taken for each type of samples.

Sensory evaluation

The sensory evaluation of biscuits replacement with dried rose was done to determine the acceptability of the product. Biscuits were evaluated for , appearance, surface color., taste, flavor, texture and overall acceptability by 13 panelists from the staff members of Food Science Department , Faculty of Agriculture, Zagazig university according to Hooda and Jood, (2005).

Biological experimental design

Albino Male rats, weighting 110-125g, were used in the study. The animals were housed in individual stainless steel cages under hygienic laboratory conditions, in the animal house of Faculty of Agriculture, Zagazig University. They were fed on the basal diet which prepared according to Ain (1993) which contain 12.5 % casein, 10 % corn oil, 4% salt mixture, 1% vitamin mixture, 5% fiber, 0.3% DL- methionine, 0.2% Choline chloride and completed to 100% by corn starch for 7 days (adaptation period), six rats were kept on basal diet and tap water along the period of experimental as negative control group (G1). Thirty rats were fed on high fat diet according to Negm (2002) to induce obesity. After induction obesity were classified into: Positive control group (G2) obese rats, groups (3, 4, 5 and 6) obese rats were fed on biscuits blended with 2.5, 5, 7.5 and 10%, respectively of dried RBP by 10% of basil diet.

During the experimental period 60 days, the quantities of diet consumed and wasted were recorded every day to calculate the food intake and body weight was recorded every week. Food efficiency ratio (FER) was calculated according to (Proll *et al.*, 1998) by using the following equation:

FER = Body weight gain / Food consumed (60 days).

Biochemical analysis of blood samples:

After 60 days, rats were fasted over night before sacrificing. Blood samples were collected from the aorta. Wassermann and EIDTA tubes were used to collect serum and

plasma samples then centrifuged for 20 minutes at 3000 rpm to separate serum and plasma. The serum and plasma were carefully separated into dry clean ependorf tubes by using a Pasteur pipette and kept frozen till analysis at -20°C. Triglycerides (Stein, 1987), total cholesterol (Young, 2001), HDL-C (Lopes *et al.*, 1977), LDL-C and VLDL-C were calculated by using the method of Friedewald *et al.* (1972) as following:

VLDL-C= Triglycerides /5

LDL-C = Total cholesterol – (HDL-C + VLDL-C)

Glucose was estimated by Trinder (1969), AST and ALT were determined according to Retiman and Frankel (1957). Uric acid Patton and Crouch, 1977, and creatinine was determined according to the methods described by Murray, 1984.

Malondialdehyde (MDA) was determined by using Biodiagnostic kit according to the method of Ohkawa (1979), determination of plasma superoxide dismutase (SOD) enzyme activity according to Roth and Gilbert (1984), the level of plasma catalase enzyme activity was assayed according to the method of Aebi (1984) and the activity of glutathione peroxidase was assayed by the method of (Chiu, *et al.*, 1976).

Histopathological examination

Tissues from liver, kidney and heart of the sacrificed rats were examined as described by Suvarna *et al*., (2013). The formalin preserved liver, kidney and heart were processed in an automated tissue processor. The processing consisted of an initial 2 step fixation and dehydration. Fixation comprising tissue immersion in 10% buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70، 90 and 100%). The tissue was initially exposed to 70% alcohol for 120 minutes followed by 90% alcohol for 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylene، followed by pure xylene for one and a half hour. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4–5 um) were stained with hematoxylin and eosin, stained sections were examined for necrosis, degeneration and any pathological changes in the liver, kidney and heat of the rat.

Statistical analysis

All statistical analyses were done by a statistical for social science package "SPSS" version 20 for microsoft windows, SPSS Inc. (Rukhin, 2012). Numerical data were expressed as mean ± SD. The levels of markers were analyzed by least significant difference (LSD). Means with different superscript letters for the same analysis are significantly different at $p \leq 0.05$ and non- significant $P>0.05$: means with the same letters.

Results and discussion

Chemical composition

Chemical composition of raw materials and biscuits fortified with RBP are shown in Table (1). The results revealed that wheat flour has higher moisture, total protein and carbohydrate than that of RBP, whereas, RBP has higher fat, ash and crude fiber compared to wheat flour. The moisture content in control biscuits increased

significantly from 4.56± 0.11to 8.29± 0.14% when DRBP is incorporated at 10% level. The increases in moisture content of biscuits containing RBP may be due to increased water absorption of dietary fiber present in RBP. During mixing, it is observed that the water required preparing the biscuit dough increased with increase of RBP ratio **(**Ajila *et al*., 2008**)**. No significant differences were found in total protein and fat contents between control biscuits and biscuits containing RBP. The ash and crude fiber contents in control biscuits increased significantly from 1.20±0.01 to 1.82±0.02% for ash and from 0.66± 0.04 to 1.99± 0.15% for crude fiber when RBP is incorporated at 10% level. This is led to increase of water absorption resulting from the interaction between hydroxyl groups of polysaccharide macromolecules present in the fiber, and water, through hydrogen bonding **(**Raymundo *et al*., 2014).

Table (1): Chemical composition of raw material and biscuits fortified with dried rose buds and petals (g/100g dry weight basis).

Chemical composition		Moisture	Total protein	Fat	Ash	Crude fiber	Carbohydrate
Wheat flour		13.76± 0.24	11.95± 0.41	0.77± 0.20	0.56±0.003	0.39± 0.03	72.57±0.77
RBP		11.57± 0.13	7.99± 0.33	3.66±0.17	5.05±0.03	8.92± 0.67	62.81±1.48
Biscuits	Control	4.56± 0.11^{e}	8.08± 0.23^{a}	12.34± 0.27^{a}	1.20±0.01^{d}	0.66± 0.04^{d}	73.15± 0.56^{a}
	2.5% RBP	5.64± 0.09^{d}	8.01± 0.27^{a}	12.43± 0.25^{a}	1.25±0.01^{c}	0.70± 0.05^{d}	71.97± 0.47^{b}
	5% RBP	6.81± 0.10^{c}	7.94± 0.30^{a}	12.51± 0.23^{a}	1.66±0.03^{b}	1.22± 0.07^{c}	69.86± 0.09^{c}
	7.5% RBP	7.17± 0.08^{b}	7.88± 0.29^{a}	12.60± 0.30^{a}	1.69±0.02^{b}	1.40± 0.10^{b}	69.26± 0.40^{c}
	10% RBP	8.29± 0.14^{a}	7.81± 0.33^{a}	12.73± 0.20^{a}	1.82±0.02^{a}	1.99± 0.15^{a}	67.37± 0.68^{d}
LSD		0.20	0.52	0.46	0.04	0.17	0.88

Means with the different superscript letters in each column are significantly at $P \leq 0.05$.

Polyphenol compounds

It was observed from Table (2) that the RBP extract is contain a numerous of polyphenol compounds. The total phenolics content in the RBP extract were found to be 6008.5± 275.06 mg/100g dry weight basis, respectively. The RBP exhibited high DPPH radical-scavenging activity with 90.46±0.94%. Furthermore, the highest concentration of the phenolic compounds in RBP extract were 1402.78± 0.010 followed by 1182.89± 0.095 then 301.72± 0.026 mg/ 100g dry weight basis for pyrogallol, ellagic and e-vanillic, repectively. The lowest concentrations were 0.38±0.006, 4.37±0.252, 4.66±0.025 and 5.74±0.015 mg/ 100g dry weight basis for cinnamic, p-coumaric, 4-amino-benzoic and caffeic, respectively. The highest concentration of flavonoid compounds present in RBP extract were for luteo.6- arabinose 8-glucose, rutin, quercetrin and hisperidin while the lowest concentrations were for rhamnetin, apig.7-glucose, rosmarinic, kaempferol and nargingenin. Kart and Çagindi (2017) determined total phenolic and flavonoid contents of dry rose tea made by using 3 different dried rose buds and 3 different dried rose petals. They found that a total phenolic and flavonoid content varies between 5.24-166.36 and 2.02-14.83 mg/200 mL tea, respectively. Also, total phenolic and flavonoid content were higher in dry petals than in dry buds. Hou *et al*. (2014) found that the total phenols, flavonoids, vit.E and vit.C were the main contributors to the antioxidant activity of rose petal extracts. The antioxidant

activity was 86.32-88.09% and peaked in the early bloom stage, and flowers in this stage had the highest functional benefits from all maturity stages.

Physical properties of biscuits

Physical properties of biscuits such as thickness, diameter and spread ratio were evaluated in Table (3) which replacement wheat flour by 2.5, 5, 7.5 and 10% of RBP caused a significant decrease in diameter from 5.62±0.01 control to 5.42cm incorporation of 10% RBP. Concerning the thickness of biscuits there were a significant gradual decrease comparable to control. The decrease in both diameter and thickness of biscuits is noticed by increasing the levels of replacement up to 10% of RBP which may be caused a dilution of gluten. Biscuits prepared from wheat flour containing 10% RBP had a hardness texture 87.73±5.7 (N) compared to 45±1.44 (N) of the control biscuits, these increase in hard ness were significant. Also, this may be refer to increase in water absorption of doughs by increasing ratio of replacement. Doughs having more water content produce an extensive (weak) gluten structure and result in harder biscuits. This results are in line with (Ajila, *et al.,* 2008) concerning decreasing in diameter the results agreement with (Abdel- Shafie and Abdulla, 2014).

Biscuits color

Color analysis of food is an important field, always related strongly to market and consumers acceptability as it controls the first impression of food product, data in Table (4) showed the influence of RBP on color values. It was observed that the *L** values were decreased significantly with the increase in the levels of RBP which control sample had the highest lightness 69.13±0.94 compared to 10% RBP enriched biscuits 34.08±0.29. The change in *a** value, which indicates the redness, gradually increased with increase the RBP level. Concerning *b** values biscuits enriched with10% RBP had the lowest b value 12.12±0.14 compared to control sample 27.43±0.38. As rose powder has red color, incorporation with wheat flour caused decrease in the lightness of the biscuits and increase redness.

Table (2): Total Phenolics, antioxidant activity and identify of phenolic and flavonoid compounds of dried rose buds and patels ethanol extract (mg/ 100g dry weight basis).

Total phenolic of dried RBP			6008.5±275.06		
Antioxidant activity %			90.46±0.94%		
Phenolic compounds	Pyrogallol	1402.78±0.010	Flavonoid compounds	Luteo.6- arabinose 8-glucose	304.67±0.030
	Gallic acid	7.70±0.015		Luteo.6- glucose 8-arabinose	2.96±0.025
	4-Amino-benzoic	4.66±0.025		Apig.6- arabinose 8-glactose	4.11±0.010
	Protocatchuic	157.77±0.026		Apig.6- rhamnose 8-glucose	4.81±0.078
	Catechein	86.32±0.021		Apig.6 glucose 8- rhamnose	15.47±0.035
	Catechol	168.00±0.195		Luteo.7-glucose	ND
	Epicatechein	52.53±0.020		Luteolin	ND
	P-OH-benzoic	109.23±0.110		Narengin	24.38±0.045
	Caffeine	20.11±0.110		Hisperidin	100.17±0.153
	Chlorogenic	60.88±0.095		Quercetin-3-O-Glucoside	ND
	Vanillic	23.55±0.055		Rutin	301.12±0.104
	Caffeic	5.74±0.015		Apig.7-O-neohespiroside	4.11±0.012
	P-Coumaric	4.37±0.252		Kaemp.3,7-dirhamoside	7.39±0.272
	Ferulic	32.63±0.035		Quercetrin	133.94±0.032
	Iso-Freulic	8.37±0.370		Rosmarinic	0.64±0.002
	Reversetrol	ND		Quercetin	6.83±0.018
	e-Vanillic	301.72±0.026		Nargingenin	1.91±0.050
	Alpha-Coumaric	34.01±0.012		Acacetin-neo.rutinoside	38.56±0.020
	Benzoic	ND		Kamp.3-(2-P-comaroyl)glucose	21.19±0.078
	Ellagic	1182.89±0.095		Hespertin	10.92±0.030
	3,4,5- methoxy-cinnamic	22.89±0.085		Kaempferol	0.66±0.057
	Coumarin	14.73±0.035		Rhamnetin	0.42±0.050
	Cinnamic	0.38±0.006		Apigenin	1.27±0.002
	Salycillic	23.94±0.015		Apig.7- glucose	0.45±0.003
				Acacetin	7.23±0.010

Table (3): Physical properties of biscuits fortified with dried rose buds and patels.

Biscuits	Diameter (cm)	Thickness (cm)	Spread ratio %	Texture (N)
Control	5.62±0.01^{a}	0.58±0.01^{a}	9.69±0.09^{d}	45.00±1.44^{c}
2.5% RBP	5.48±0.01^{b}	0.55±0.01^{b}	9.96±0.12^{cd}	60.38±8.02^{b}
5% RBP	5.32±0.06^{d}	0.53±0.01^{c}	10.04±0.22^{bc}	63.79±6.88^{b}
7.5% RBP	5.47±0.01^{b}	0.53±0.00^{c}	10.33±0.01^{b}	79.70±2.33^{a}
10% RBP	5.42±0.01^{c}	0.48±0.02^{d}	11.29±0.39^{a}	87.73±5.70^{a}
LSD	0.048	0.016	0.381	10.022

Means with the different superscript letters in each column are significantly at $P \leq 0.05$

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Table (4): Color characteristics of biscuits fortified with dried rose buds and petals

Biscuits	*L* *	*a* *	*b* *
Control	69.13±0.94^{a}	2.75±0.65^{c}	27.34±0.38^{a}
2.5% RBP	46.20±0.37^{b}	6.46±0.48^{b}	17.66±0.37^{b}
5% RBP	39.95±0.25^{c}	6.91±0.37^{b}	14.81±0.18^{c}
7.5% RBP	35.08±0.65^{d}	8.03±0.06^{a}	13.08±0.56^{d}
10% RBP	34.08±0.29^{d}	8.61±0.21^{a}	12.12±0.14^{e}
LSD	1.024	0.742	0.654

L*: lightness, a*: redness, b* yellowness.

Means with the different superscript letters in each column are significantly at $P \leq 0.05$.

Sensory evaluation results of biscuits:

The web chart for mean sensory evaluation scores of biscuits containing RBP showed that control biscuits had the highest score for all the sensory attributes followed by biscuits containing 2.5 % RBP then 5% RBP (Fig. 1). A significant difference ($p \leq 0.05$) was found in the color between biscuits containing 5, 7.5 and 10% RBP and control. This is due to that incorporation of RBP caused relatively dark color. The darkness increased with increasing the RBP% in biscuits, hence was reflected on *L* values (Table 4) which may be due to the non-enzymatic browning reactions **(**Martínez-Girón *et al*., 2017). A significant decrease ($p \leq 0.05$) in the color scores were observed with increasing the RBP% in biscuits. This may be attributed to the slight bitter taste which due to high polyphenol content (Ajila *et al*., 2008). No significant difference was found in the odor between biscuits containing 2.5, 5 and 7.5% RBP and control, because RBP is used as flavoring agents of foods. A significant decrease in the texture was found with increasing the RBP% in biscuits. This may be due to hardness of biscuits containing RBP compared to the control with increasing of RBP%. Also, the increase in the hardness was substantiated by the increase in the hardness value as measured using a texture analyzer (Ajila *et al*., 2008). A significant decrease in overall acceptability was observed with increasing the RBP% in biscuits. Finally, all biscuits containing RBP were acceptable by panelists.

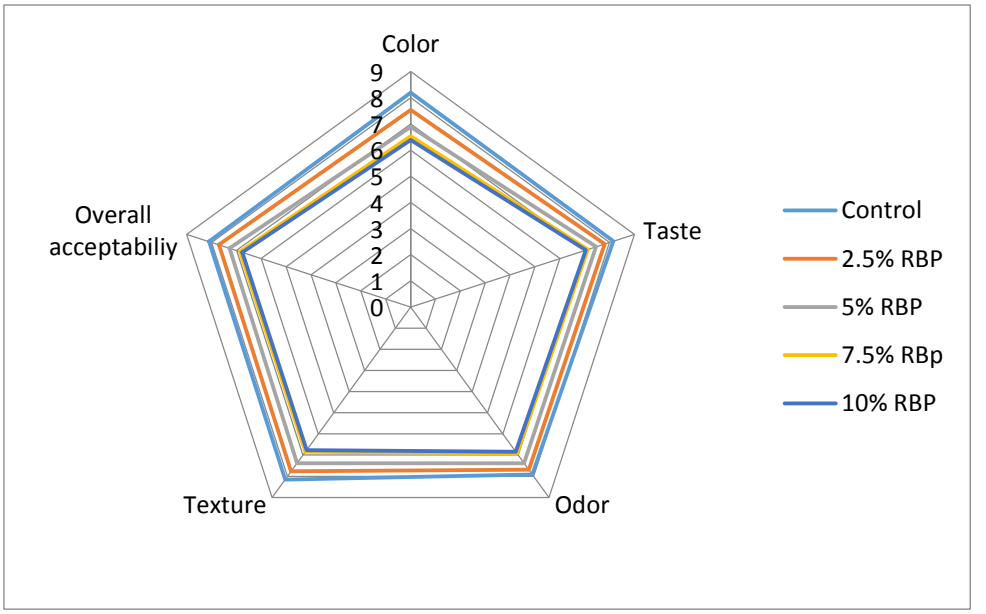

Fig. (1): Web chart for mean sensory evaluation scores of biscuits fortified with dried rose buds and petals.

Body weight gain and food intake of obese rats

Effect of fed obese rats on biscuits supplemented with different levels of RBP on body weight gain, food intake and feed efficiency ratio are shown in Table (5) it was observed that, positive control group had the highest BWG, food intake and feed efficiency ratio compared to negative control group which the mean value of body weight gain, food intake and FER were increased significantly at ($P \leq 0.05$) in positive control (obese rats) compared to negative control group. Whereas, obese rats fed on biscuits blended with RBP caused a significant decrease compared to positive control group but these decrease were gradual in these parameters. In general, fed obese rats on biscuits fortified with 5, 7.5 and 10% of RBP led to significant decrease in BWG, FI and FER compared with the rat fed on high fat diet. Dried RBP are rich in bioactive compounds such as phenolic, flavonoids, anthocyanine and fiber that (which contributes

effectively and significantly in weight loss) can help in burning fats and weight loss. These results are matched with those obtained by Sayed (2014) and El- Shaer *et al.* (2016) who revealed that the rats fed on high fat diet had a significant increase in food intake, body weight gain and feed efficiency ratio compared with negative control group which fed on basal diet.

Table (5): Effect of biscuits fortified with dried rose buds and patels on body weight gain, food intake and FFR of obese rats.

Groups		Body weight gain (g/60 day)	Food intake (g/day)	FER
Control (-ve)		74.82±4.13^{c}	22.10±1.34^{d}	0.056±0.002^{b}
Obese rats	Control (+ve)	129.75±2.52^{a}	28.93±0.69^{a}	0.075±0.001^{a}
	2.5% RBP	88.23±1.77^{b}	26.48±0.96^{b}	0.056±0.001^{b}
	5% RBP	63.71±1.63^{d}	24.20±1.15^{c}	0.044±0.002^{c}
	7.5% RBP	57.13±5.04^{e}	22.93±0.84^{cd}	0.041±0.003^{c}
	10% RBP	49.20±4.06^{f}	22.29±0.18^{d}	0.037±0.002^{d}
LSD		6.12	1.66	0.003

Means with the different superscript letters in each column are significantly at $P \leq 0.05$.

Lipid profile of obese rats

Recently, the bioactive components in foods and functional foods have become popular and been considered as complementary or alternative therapeutic agent to treat or manage chronic disease such as obesity and diabetes. Therefore more of herbs and dietary supplements are widely used to manage obesity which may be an excellent strategy for developing future effective, safe anti-obesity (Connell, 2001 and Mayer, *et al.*, 2009). As shown in Table (6) feeding obese rats on biscuits blended with RBP caused a significant improvement on lipid profile which the rate of effective was increased by increasing the ratio of RBP in biscuits. Obese rats (positive control group) had high levels of TG, TC, LDL and VLDL compared to negative control group. Also, it could be observed that positive control group appears a significant decrease at ($P \leq$ 0.05) in the mean values of HDL 38.71±6.25 mg/dl compared to negative control 62. 37± 2.16 mg/dl and there was no significant differences between obese rats fed on biscuits blended with 10% RBP in all lipid parameters compared to negative control. the obtained results are in line with (Shalaby and El-Shourbagy, 2014) who found that peanut skin biscuits caused a significant decrease in serum total cholesterol, triglyceride and low density lipoprotein and a significant increase in the level of HDL. Also, these results are in agreement with those obtained by Ravi and Kumar (2013) reported that *Moringa oleifera* leaves were found to lower the serum cholesterol, TG, VLDL and LDL, but were found to increase the HDL.

Table (6): Effect of biscuits fortified with dried rose buds and petals on lipid profile of obese rats.

Groups		Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control (-ve)		82.90±3.08^{e}	102.36±2.66^{de}	62.37±2.16^{a}	23.41±2.97^{d}	16.58±0.62^{e}
Obese	Control (+ve)	168.81±8.45^{a}	160.65±2.04^{a}	38.71±6.25^{d}	88.18±6.64^{a}	33.76±1.69^{a}

	2.5% RBP	149.19±2.32^{b}	143.37±3.56^{b}	45.39±2.94^{c}	68.14±1.83^{b}	29.84±0.46^{b}
	5% RBP	113.41±5.06^{c}	124.05±3.61^{c}	52.08±1.05^{b}	49.29±4.94^{c}	22.68±1.01^{c}
	7.5% RBP	99.36±3.43^{d}	105.55±4.30^{d}	58.15±3.91^{ab}	27.53±5.49^{d}	19.87±0.69^{d}
	10% RBP	89.03±4.95^{e}	99.11±2.28^{e}	60.47±3.34^{a}	20.84±1.67^{d}	17.80±0.99^{e}
LSD		8.841	5.653	6.490	7.736	1.768

Means with the different superscript letters in each column are significantly at $P \leq 0.05$.

Malonaldehyde (MDA) and antioxidant enzymes of obese rats

Table (7) shows the effect of feeding obese rats on biscuits blended with RBP on the levels of plasma antioxidant enzymes and MDA. Obese rats (positive control group) had a significant lower in SOD, CAT and GPx enzymes compared to negative and treated groups. Also, positive control group recorded high significant increase in the levels of MDA compared to negative control group. Meanwhile, fed obese rats on biscuits fortified with different levels of RBP caused a significant improvement in the levels of antioxidant enzymes and MDA compared to positive control. Whereas, fed obese rats on biscuits fortified with biscuits supplemented with 10% RBP is caused more activation for SOD, CAT, GPx enzymes, but with no significant differences compared to negative control group. It was observed that dried rose buds and patels had more bioactive compounds which led to activate the antioxidant enzymes and decrease from lipid peroxidation which cause a significant decrease in levels of MDA. The obtained results are in line with Abo-Raya *et al.* (2013) who found that oral administration of red ginseng extract to obese diabetic rats significantly increased activities of SOD, GPx and CAT antioxidant enzymes.

Table (7): Effect of biscuits fortified with dried rose buds and petals on the levels of MDA and antioxidant enzymes of obese rats.

Groups		MAD (nmol/ L)	SOD (u/ml)	CAT (u/ml)	GPx (u/ml)
Control (-ve)		15.44±4.71^{d}	342.40±42.68^{a}	396.96±8.56^{a}	276.38±23.37^{a}
Obese rats	Control (+ve)	36.32±4.08^{a}	170.84±20.32^{d}	211.10±10.11^{e}	152.82±45.67^{c}
	2.5% RBP	25.40±1.81^{b}	227.56±10.71^{c}	282.78±12.68^{d}	194.62±37.88^{b}
	5% RBP	20.14±2.24^{c}	303.38±10.23^{b}	310.22±23.64^{c}	224.26±10.72^{b}
	7.5% RBP	17.48±1.3^{cd}	334.82±9.52^{a}	357.42±22.73^{b}	270.34±19.45^{a}
	10% RBP	14.24±3.69^{d}	355.46±23.51^{a}	403.46±11.98^{a}	301.42±5.73^{a}

LSD	4.25	29.62	21.01	36.11

Means with the different superscript letters in each column are significantly at $P \leq 0.05$.

Glucose level of obese rats

Glucose levels of obese rats fed on biscuits fortified with dried rose buds and patels compared with negative and positive control groups are shown in Fig. (2). It is evident that there were significant differences ($p \leq 0.05$) in glucose levels between negative control group and positive control group. The mean values were 99.14± 1.02 and 206.67± 24.88 mg/ dl, respectively. While, there were non-significant differences ($p > 0.05$) between negative control and obese rats fed on biscuits fortified with 5 and 7.5% RBP. The highest reduction of glucose level recorded with obese rats fed on biscuits fortified with 10% RBP (87.63± 12.44 mg/ dl). These results agree with epidemiological studies which have assured a strong positive relation between obesity and risk of developing type 2 diabetes. The risk of diabetes increases by about 9% per kilogram increase in body weight (Dal and Sigrist, 2016). Ju *et al.* (2014) stated that red rose flowers extracts have long-term anti-diabetic effects, and that effect is independent of polyphenol levels in the extract.

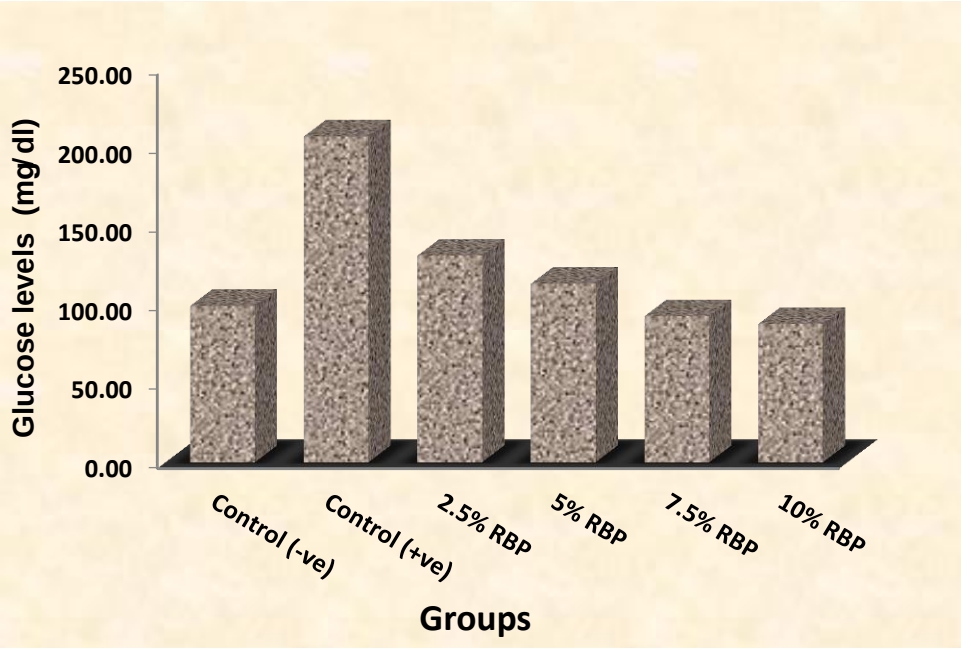

Fig. (2): Glucose levels of obese rats fed on biscuits fortified with dried RBP compared with negative and positive control groups.

Kidney and liver function of obese rats:

Data given in Table (8) show the effect of biscuits fortified with RBP on kidney and liver functions of obese rats. It was observed that the positive control group showed a significant increase in ALT enzyme to be 100.28±22.08 U/L compared to negative control group (69.35±10.05 U/L). Also, the negative control group had lower ($P \leq 0.05$) in AST activity than positive control group. Moreover, feeding obese rats on biscuits fortified with 2.5, 5, 7.5 and 10% RBP caused a significant reduction in ALT and AST enzymes activity compared to positive control group. Ju *et al.* (2014) stated that the rose flowers extracts have a significant effect on reduction of ALT and AST levels in blood of diabetic rats.

Concerning to kidney function in the same Table, it is evident that there were significant differences in serum urea and creatinine levels between negative control

group and positive control group. Feeding obese rats on biscuits fortified with 2.5, 5, 7.5 and 10% RBP resulted in a significant decrease for serum urea and creatinine levels to be (29.00±4.24, 25.89±2.10, 24.19±3.80 and 23.84±4.67 mg/dl) and (1.04±0.22, 0.92±0.11, 0.94±0.07 and 0.88±0.07 mg/dl) respectively, compared to positive control group (35.87±3.09 and 1.21±0.08 mg/dl) respectively. Furthermore, there were non-significant differences between obese rats fed on biscuits fortified with 5, 7.5 and 10% RBP and negative control group.

Table (8): Effect of biscuits fortified with dried rose buds and petals on kidney and liver functions of obese rats.

Groups		Liver function		Kidney function	
		ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control (-ve)		69.35±10.05^{bc}	93.33±12.88^{b}	25.22±2.30^{bc}	0.95±0.13^{b}
Obese rats	Control (+ve)	100.28±22.08^{a}	149.92±38.46^{a}	35.87±3.09^{a}	1.21±0.08^{a}
	2.5% RBP	85.41±17.96^{ab}	115.11±18.58^{b}	29.00±4.24^{b}	1.04±0.22^{b}
	5% RBP	65.96±15.84^{c}	100.15±3.52^{b}	25.89±2.10^{bc}	0.92±0.11^{b}
	7.5% RBP	60.21±7.76^{c}	98.09±8.74^{b}	24.19±3.80^{c}	0.94±0.07^{b}
	10% RBP	59.64±6.55^{c}	91.00±8.80^{b}	23.84±4.67^{c}	0.88±0.07^{b}
LSD		18.95	24.75	4.57	0.16

Means with the different superscript letters in each column are significantly at $P \leq 0.05$.

Histopathological examination

Liver

Normal hepatic structures (portal trade, central vein, sinusoids and hepatocytes) were noticed in negative control group. In positive control group (obese rats), congestion of the major of blood vessels was commonly observed and widening of central vein. Multiable portal and/or interstitial round inflammatory aggregates beside mild fibroblasts proliferations and in sometimes become minute portal fibrous threads which extend to in the interlobular septa. Focal sub-capsular and or interstitial coagulative necrosis and some sections revealed the hepatocytes suffered acute cell swelling. Scattered a few number of hepatocytes were apoptotic. Some sections revealed variable sizes of fatty changes. Mild congested of hepatic blood vessels and sinusoids were detected in obese rats fed on biscuits fortified with 2.5% RBP.

The majority of hepatic lobules showed within normal while 60% of the suffered from acute cell swelling. Minute focal round cells infiltration could be seen in some portal areas. Mild portal fibrosis and periportal area showed a few steatosis. Focal apoptosis hepatocytes were also seen. Hyperplastic of kupffer cells was noticed. In obese rats fed on biscuits fortified with 5% RBP, the majority of hepatic parenchyma showed within the normal histomorphological structures. Mild portal inflammatory cells infiltrations adjacent of widening of sinusoids. Some hepatic portal area suffered from mild edema, fibrosis and proliferated of bile epithelium with formation canaliculi. Widening of central vein and kupffer cells hyperplasia were noticed. Some hepatocytes showed within double nuclei (diplocytes). Capsular thickening due to fibrosis and inflammatory cells infiltration were prominent. The majority of the hepatic structures showed within the normal central vein and hepatic cords and portal trades. Minute

subscapular, periportal and /or interstitial inflammatory cells infiltrations were seen in obese rats fed on biscuits fortified with 7.5% RBP. Obese rats fed on biscuits fortified with 10% RBP were caused improvement and normal healthy hepatic lobules (Fig. 3).

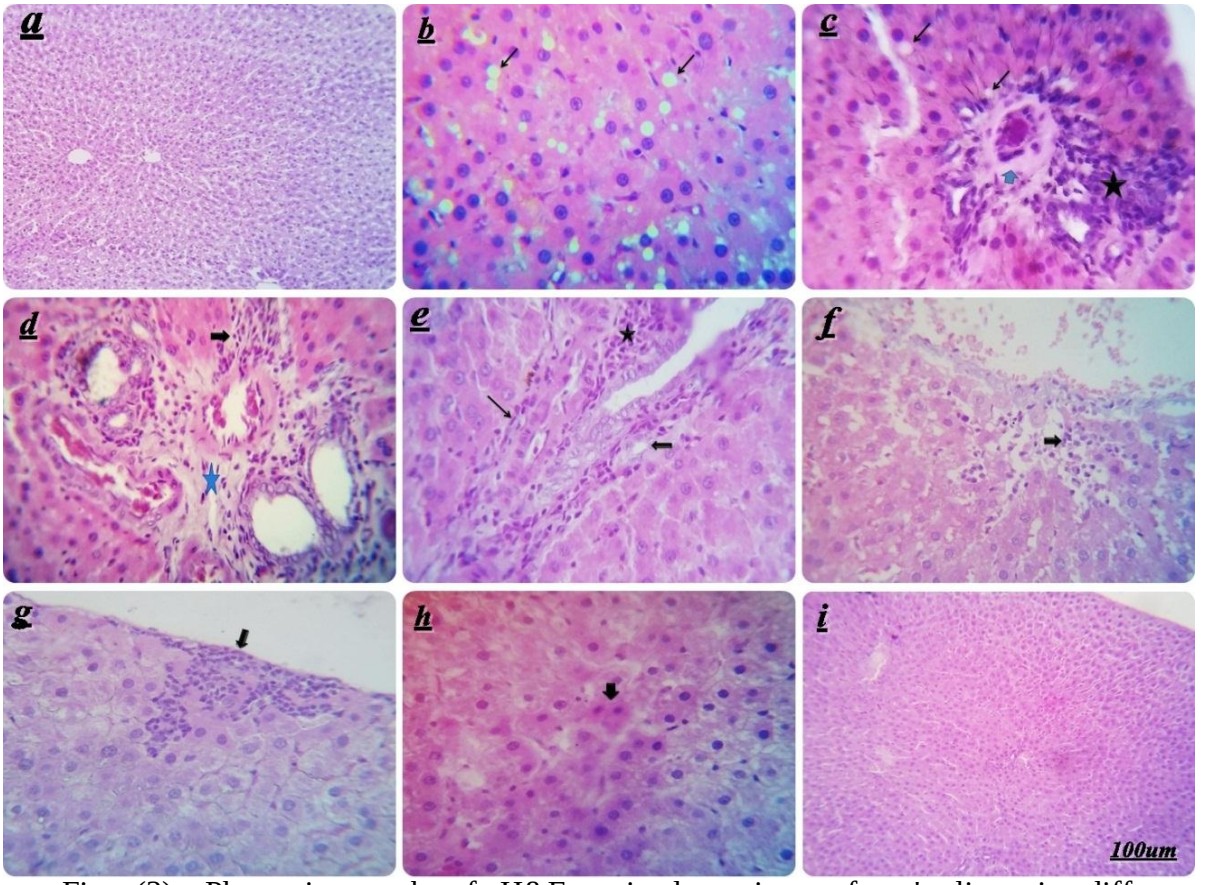

Fig. (3): Photomicrograph of H&E stained sections of rat's liver in different experimental groups (from a to i) showing normal hepatic parenchyma in control (a) and 10% RBP (i) groups. Perivascular edema (thick arrow), steatosis (thin arrows), portal lymphocytic aggregation (star) beside portal fibrosis (star) infiltrated with inflammatory cells (thick arrow) and proliferation bile ducts in Obesity group (b, c and d). Portal inflammatory cells aggregation (star), hyperplastic bile epithelium with newly formed bile ductules (thick arrow) and numerous of spindle cells (thin arrow) adjacent normal hepatocytes in 2.5% RBP group (e). Mild edema infiltrated by numerous inflammatory cells (arrow), beside normal hepatic cords and sinusoids beside (f) and mild subscapular inflammatory cells (arrow), beside healthy hyperchromatic hepatocytes (g) in 5% RBP group. Apparently normal hepatic parenchyma with a few apoptotic cells (arrow) is seen in 7.5% RBP group (h). Scale bar 100um.

Kidney

The histopathological examination of rat's kidney is shown in Fig. (4). Normal renal cortex and medulla structures (glomeruli and tubules) were seen in negative control group and obese rats fed on biscuits fortified with 10% RBP. While hyper-cellularity and congested tuft capillaries, cystic dilatation of renal tubules, casts in some renal tubules, some renal tubules showed destructed, necrosis and apoptosis were detected in positive control group. Mild interstitial hemorrhage was also seen beside multiable periglomerular and interstitial inflammatory cells mainly lymphocytes aggregations. In obese rats fed on biscuits fortified with 2.5% RBP, it was observed small focal interstitial inflammatory cells aggregations in some sections but others

showed interstitial hemorrhages. Necroses of some glomeruli were not prominent. Casts or portions materials (hyaline or granular) were seen in some areas adjacent necrotic renal tubules. Perivascular edema, inflammatory cells infiltrations and sometimes large area of fibrosis in renal medulla area were detected. Cystic dilatation in the major renal tubules and mild interstitial hemorrhages were seen in obese rats fed on biscuits fortified with 5% RBP. Also, fibrous threads were seen in the corticomedullary junctions. Some glomeruli revealed hyper-cellularity and thickening of visceral and partial layers. The remaining renal parenchyma was normal. In obese rats fed on biscuits fortified with 7.5% RBP, the renal parenchyma showed within the normal structures and some portionus materials showed within the lumina of some tubules. Mild perivascular edema was also seen.

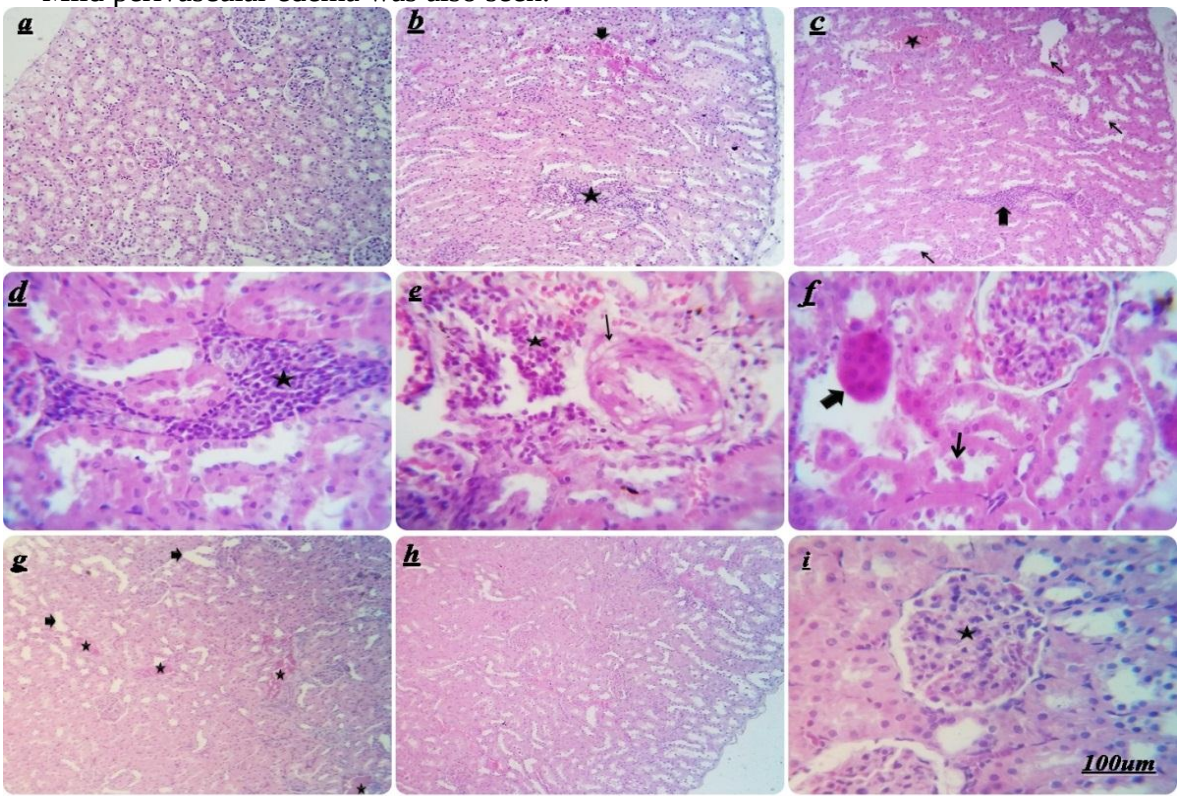

Fig. (4): Photomicrograph of H&E stained sections of rat's kidney in different experimental groups (from a to i) showing normal renal parenchyma in control group (a), normal glomeruli with hyper-cellularity adjacent normal renal tubules in 7.5% and 10% RBP groups (h and i). Interstitial inflammatory cells aggregations (star) among degenerated tubules and subscapular hemorrhages (thick arrow) in Obesity group (b). Cystic dilatation of renal tubules (thin arrows), focal interstitial inflammatory cells aggregations (thick arrow) and mild interstitial hemorrhages (star) (c) beside interstitial lymphocytic aggregations (star) among the degenerated renal tubules (d), perivascular edema, hemorrhages and lymphocytic aggregation (star), endotheliosis and vacuolar media of the renal artery (thin arrow) (e) while many castes in lumina of some renal tubules (thin arrows), focal renal necrosis (thick arrow) (f) are seen in 2.5%RBP group. Mild congested interstitial blood vessels (stars) with slightly dilated renal tubules (thick arrows) beside normal renal parenchyma (g) in 5% RBP group. Scale bar 100um.

Heart

The histopathological examination of rat's heart is shown in Fig. (5). Normal cardiac structures (pericardium, cardiac muscles and cardiac blood vessels) were seen in negative control group. Congested large blood vessels and some of them were endotheliosis and vacuolated media, intramuscular edema with hyalinized myocardial muscles. Extravasated erythrocytes in intramuscular spaces were seen in positive control group. In obese rats fed on biscuits fortified with 2.5% RBP, myocarditis was prominent mainly lymphocytes adjacent hyalinized myocardial fibers and some sections revealed microvacules in myocardial cells. Mild pericarditis represented by fibrous edema infiltrated by inflammatory cells mainly lymphocytes and macrophages. Also, mild congested blood vessels and interstitial round cells infiltration were seen in obese rats fed on biscuits fortified with 5% RBP. Normal myocardial muscles were seen in some sections of rat's heart fed on biscuits fortified with 7.5% RBP. While, other were showed partially hyalinized fibers with a few round cells infiltrations. Obese rats fed on biscuits fortified with 10% RBP were caused improvement and normal healthy cardiac muscles with still some of them suffered partially hyalinized fibers.

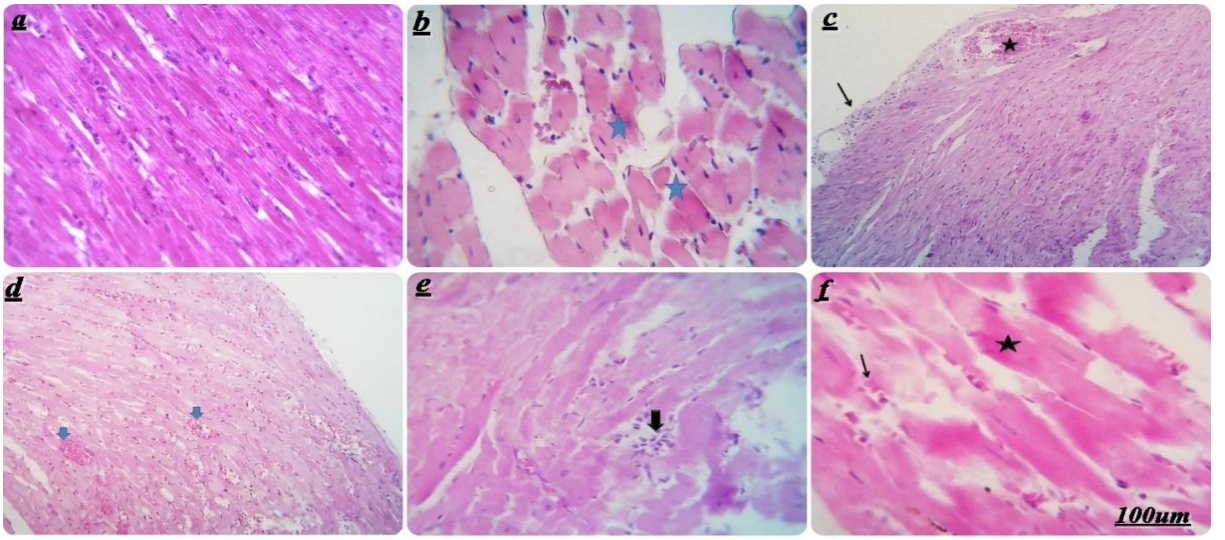

Fig. (5): Photomicrograph of H&E stained sections of rat's heart in different experimental groups (from a to f) showing normal myocardial fibers in control group (a). Hyalinized myocardial fibers (star) and intramuscular extravasated erythrocytes in Obesity group (b). Mild pericarditis (arrow) and mild congested cardiac blood vessels (star) in 2.5% RBP group (c). Mild congested cardiac blood vessels (arrows), beside normal cardiac muscles fibers in 5% RBP group (d). Partially hyalinization of the myocardial fibers with minute round cells (arrow) is seen in 7.5% RBP group. While normal myocardial muscle fibers with slightly hyalinization of a few myocardial fibers (arrow) in 10% RBP group (f). Scale bar 100um.

Conclusion

From the current study, it could be concluded that the dried RBP is rich with polyphenol compounds and high antioxidant activities. In addition, Feeding rats on biscuits fortified with RBP showed a significant enhancing in lipid profile, liver enzymes lowering, improvement in kidney function parameters and glucose levels

compared to positive control group. Biscuits fortified with RBP are a healthy and functional food for treatment of obesity. So, this study recommends using of dried RBP for the preparation of biscuits and other functional food products for treating obese patients. Further studies are needed to investigate the role of RBP in prevention or treating from other diseases.

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تقييم النشاط المضاد للسمنة للبسكويت المدعم بزر الورد المجفف على الفئران البيضاء

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الملخص

يهدف هذا البحث إلى دراسة تقييم تأثير استبدال دقيق القمح بزر الورد الأحمر المجفف بنسب ٢.٥ و ٥ و ٧.٥ و ١٠٪ على التركيب الكيميائي والخصائص الفيزيائية والحسية للبسكويت وتأثير استهلاك البسكويت على الفئران المصابة بالسمنة، وقد تم استخدام ستة وثلاثون من ذكور الفئران البيضاء تم تقسيمهم الى ست مجموعات متساوية ،استخدمت إحدى هذه المجموعات كمجموعة ضابطة سالبة أما باقى المجموعات فتم تغذيتهم على وجبات عالية الدهن لمدة شهر لإصابتهم بالسمنة، ثم استخدمت إحدى هذه المجموعات كمجموعة ضابطة موجبة أما باقى المجموعات تم تغذيتهم على الوجبة الأساسية المضاف لها ١٠٪ من البسكويت المدعم بزر الورد المجفف بنسب ٢.٥ و ٥ و ٧.٥ و ١٠٪ لمدة ٦٠ يوم. وأوضحت النتائج أن مستخلص زر الورد يحتوي على العديد من المركبات الفينولية والفلافونويدية وأظهر كذلك نشاطا عاليا كمضاد للأكسدة ، ووجد ان البسكويت المحتوى على ١٠٪ زر ورد مجفف قوامة كان أكثر صلابة بمعدل (٨٧.٧٣ ± ٥.٧ نيوتين) مقارنة بالبسكويت الكنترول (٤٥.٠٠ ± ١.٤٤ نيوتين)، وحصلت كل أنواع البسكويت على قبول حسى ولكن البسكويت المحتوى على ٢.٥% زر ورد كان أكثر قبولاً بعد الكنترول يليه البسكويت المحتوى على ٥% ثم ٧.٥ و ١٠٪، و أدت تغذية الفئران المصابة بالسمنة على البسكويت المدعم بزر الورد بنسب ٧.٥ و ١٠٪ إلى حدوث إنخفاضا معنويا فى مستوى كلا من الجليسريدات الثلاثية والكوليسترول الكلى وLDL-cholesterol والجلوكوز وإنزيمات الكبد (AST و ALT) وتحسن وظائف الكلى مقارنة بالمجموعة الضابطة الموجبة. أيضا حدث إنخفاض معنوى فى مستوى المالوندالدهيد (MDA) وزيادة فى الانزيمات المضادة للأكسدة. ومن نتائج هذه الدراسة يمكن التوصية باستهلاك البسكويت المدعم بزر الورد المجفف ضمن النظام الغذائي اليومي للمرضى المصابون بالسمنة لتحسين مستوى الدهون بالجسم وفقد الوزن.

الكلمات المفتاحية: زر الورد، البسكويت، السمنة، دهون الدم، الانزيمات المضادة للأكسدة.