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Feeding On Some Selected Food Processing By-Products To Improve The Obesity Disease Complications In Rats

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Abstract: The present study aims to investigate the effectiveness of selected food processing by-products mixed in loaves bread in modulating obesity parameters and its complications in obese rats. Forty two (160-170g per each), were divided into two main groups, the first group (Group 1, 6 rats) still fed on basal diet and the other main group (36 rats) was feed with diet-induced obesity (DIO) for 8 weeks which classified into sex sub groups as follow: group (2), fed on DIO as a positive control; groups (3-7), fed on DIO containing 5 % potato peel powder (PPP), cauliflower leaves powder (CLP), red onion skin powder (ROSP), mango peel powder (MPP) and their mixture, respectively. Feeding of rats on diet induced obesity (DIO) for eight weeks leads to increase the bodyweight (BW) than the control group which recorded 183.27 and 221.50 % of baseline, respectively. Replacement of wheat flour with 5% of PPP, CLP, ROSP, MPP and their mixture induced significant decreasing on BW of the obese rats which recorded 205.47, 210.48, 196.91, 198.61 and 191.09 % of baseline, respectively. Biochemical analysis data indicated that obesity induced a significant increased in blood lipids profiles (TG, 32.52%; TC, 23.04% and LDL, 51.78%) ($p \leq 0.05$), liver functions (ALT, 33.65% and AST, 32.54%), kidney functions (uric acid, 33.68% and creatinine, 29.62%) and serum glucose (38.63%) compared to normal controls. Also, some immunological parameters (albumin level and protease activity) in serum of obese rats were significant decreased ($p \leq 0.05$). Feeding on 5% of PPP, CLP, ROSP, MPP and their mixture exhibited a significant improvement ($p \leq 0.05$) in all of these parameters by different rates. The higher amelioration effects were recorded for the by-product mixtures treatment followed by ROSP, MPP, PPP and CLP, respectively. In conclusion, these findings provide a basis for the use of the selected food processing by-products and also have important implications for the prevention and early treatment of obesity.

Keywords: potato peel, cauliflower leaves, onion skin, mango peel, liver functions, kidney functions, blood lipids profile, serum glucose.

Introduction

Obesity is an abnormal accumulation of body fat, usually 20% or more over an individual's ideal body weight (Beers *et al.*, (2004). The prevalence of overweight and obesity is commonly assessed by using body mass index (BMI), defined as the weight in kilograms divided by the square of the height in meters (kg/m2). A BMI over 25 kg/m^2 is defined as overweight, and a BMI of over 30 kg/m^2 as obese. These markers provide common benchmarks for assessment, but the risks of disease in all populations can increase progressively from lower BMI levels (Caterson, 2009 and Muñoz-Garach *et al.*, 2016).

According to the World Health Organization, there are more than one billion overweight adults in the world. At least 300 million of them are clinically obese (WHO, 2006) and of these about 115 million come from developing countries (WHO and Dini , 2006). Furthermore, in the past 20 years, the rates of obesity have tripled in developing countries (Hossain *et al.*, 2007). Egypt, a developing country, is undergoing rapid urbanization changes. This has a direct impact on its people's dietary habits and physical activity patterns. According to national studies, it is common to skip meals and to replace them with daily snacks, and most of these snacks are high in calories and low in nutrients. So, Egypt appeared in No. 8 ranking among the countries of the world where obesity - adult prevalence rate, 30.3% (http://www.indexmundi.com /egypt/ obesity adult_prevalence rate. html).

Excessive body weight is associated with various diseases, particularly cardiovascular diseases, diabetes mellitus type 2, obstructive sleep apnea, certain types of cancer, osteoarthritis and asthma. (Aronne and Segal, 2003; Caterson, 2009 and Alexopoulos *et al.*, 2016). Also, obesity increases the risk of many physical and mental conditions. These co-morbidities are most commonly shown in metabolic syndrome, a combination of medical disorders which includes: diabetes mellitus type 2, high blood pressure, high blood cholesterol, and high triglyceride levels (Grundy, 2004). Complications are either directly caused by obesity or indirectly related through mechanisms sharing a common cause such as a poor diet or a sedentary lifestyle (Bray, 2004).

As a result, obesity has been found to reduce life expectancy (Haslam and James, 2005). In this direction, Mokdad *et al.,* (2004) reported that obesity is one of the leading preventable causes of death worldwide. Large-scale American and European studies have found that mortality risk is lowest at a BMI of 20–25 kg/m^2 in non-smokers and at 24–27 kg/m^2 in current smokers, with risk increasing along with changes in either direction. A BMI above 32 kg/m^2 has been associated with a doubled mortality rate among women over a 16-year period. In the United States obesity is estimated to cause 111,909 to 365,000 deaths per year, while 1 million (7.7%) of deaths in Europe are attributed to excess weight. On average, obesity reduces life expectancy by six to seven years, a BMI of 30–35 kg/m^2 reduces life expectancy by two to four years, while severe obesity (BMI > 40 kg/m2) reduces life expectancy by ten years (Whitlock *et al.*, 2009)

Industrialization of agriculture in the Arab world represent a large proportion of waste was estimated at 18.14 million tonnes per year and represent remnants of fruit and vegetables manufacture about 6.14% of this amount (http://elasaala.blogspot.com/2012/01/blog-post_2703. html). Processing of fruits and vegetables are resulting in high amounts of waste materials/py-products such as peels, seeds, stones, meals etc. It is well known that agroindustrial by-products are rich in dietary fibers, some of which contain appreciable amounts of colorants, antioxidant compounds or other substances with positive health effects, while some of them, like the oilseed meals, are rich in proteins (reviewed in Vasso and Constantina, (2007). Some major source of food by-products are potatoes, cauliflower, onion and mango some of the most popular vegetables and fruits.

Potato (*Solanum tuberosum* L.) is the largest vegetable crop worldwide, amounting to approximately 320 million metric tons annually (FAO, 2005). Processing of potatoes (mainly for the production of chips, French fries, and dehydrated products) has presented a steady increase during the last decades, exceeding considerably the amount of the vegetable consumed as fresh (Kadam *et al.*, 1991; Schieber *et al.*, 2001 and Vasso and Constantina, 2007). Solid waste generated during processing consists mostly of potato peels but also contains green, immature, and cull potatoes and amounts to 15–45% depending on the procedure applied (Schieber *et al.*, 2001). Mango (*Mangifera indica* L.) is one of the major tropical fruits and the world's annual production is 25 MMT (FAO, 2004). As mango is a seasonal fruit, mango fruits are processed into various products such as puree, nectar, leather, pickles, canned slices, etc., which have worldwide popularity (Loelillet, 1994). Mango peel considered as a waste by-product which obtained it during processing of mango, huge amount of peel is generated, and its disposal is a major problem and causes environmental pollution. Peel constitutes about 15–20% of mango fruit. Onions (*Allium cepa* L.) are the second most important horticultural crop worldwide, after tomatoes, with current annual production around 66 million tonnes. Over the past 10 years, onion production has increased by more than 25% (FAO, 2008). The main onion waste include onion skins, two outer fleshy scales and roots generated during industrial peeling and undersized malformed or damaged bulbs (Benitez *et al*., 2011). Cauliflower *Brassica L. var. Botrytis* belongs to cruciferous family *Cruciferae* (*Brassicaceae*), which comprises also: cabbage, broccoli, Brussels sprouts, turnip, Swedish turnip. Cauliflower leaves considered as a waste by-product which obtained it during processing (freezing and cooking) of Cauliflower, huge amount of leaves is generated, and its disposal is a major problem and causes environmental pollution (Ali, 2013). Leaves constitutes about 40-50% of cauliflower fruit.

Many studies reported that all of the previous by-products are rich sources of bioactive compounds including vitamins (C, E and β-carotene), polyphenols, organo-sulphur compounds, dietary fiber etc (Schieber *et al.*, 2001; Pushp and Marleny, 2011; Sabeena *et al.*, 2012 and Elhassaneen *et al.*, 2016). Varied bioactive components at different levels may be responsible for the offered health protection. A number of experiments indicate that such by-products added to laboratory animals diet had positive effects on serum lipid profile, liver and kidney functions and serum glucose (Coskun *et al.*, 2005; Gorinstein *et al*., 2006; Taing, *et al.*, 2012 and Matsunaga *et al*., 2014). In the present study we will try to open new avenue for extending the using of such four food processing by-products (potato peel, cauliflower leaves, onion skin, and mango peel) in therapeutic nutritional applications through mixing them in loaves bread to improve the obesity disease complications in rats

Material and Methods

Materials

Wheat flour: Variety Giza 155 wheat (*Triticum vulgare*) was obtained from the Egyptian local markets during the 2014 harvesting period. The collected samples was transported to the laboratory and stored immediately on the refrigerator at 0 ^{0}C until using in preparation of flour.

Food by-products: Red onion skin (ROS) was obtained from the New Beni Suef company for Preservation, dehydration and Industrialization of Vegetables, Beni Suef Elgudida City, Nile East, Beni Suef, Egypt; potato peel (PP) from *SFCO* For Manufacturing & Export Agricultural Products, El Negila, Kom Hamada, Behira Government , Egypt. Mangoes (*Mangifera indica* L. cv Copania) fruits were obtained from a local farm, Ismalia Road (El-Salhia), Egypt and used for mango peels preparation. Cauliflower (*Brassica oleracea* L. cv Copania) leaves were obtained from Damietta local markets, Menoufiya Governorate, Egypt during the 2014 harvesting period. The collected samples was transported to the laboratory and used immediately for cauliflower peels preparation.

Salt, bicarbonate soda, yeast and shortening were purchased from the Egyptian local markets.

Methods

Preparation of food by-products peel powder

Mango peel powder (MPP): Unripe mango peel were soaked in 0.1% sodium metabisulphite solution for 30 min, washed, sliced and dried in two stages at 60 ^{0}C for 12 and 40 ^{0}C for 12 hours in hot air oven (AFOS Mini Smoker, England). This is followed by milling with grinder (Retsch Micro Universal Bench Top Grinder, Germany) to produce the respective flour types.

Red onion skin powder (ROSP) and potato peel powder (PPP): Red onion skin and potato peel were washed and then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 55 ^{0}C for 14. The dried peels were ground into a fine powder in high mixer speed (Moulinex Egypt, Al-Araby Co., Egypt). The material that passed through an 80 mesh sieve was retained for use.

Cauliflower leaves powder (CLP): Cauliflower leaves were washed and then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at two stages 50 ^{0}C for 6 hrs followed by 40 ^{0}C for 10 hrs. The dried peels were ground into a fine powder in high mixer speed (Moulinex Egypt, Al-Araby Co., Egypt). The material that passed through an 80 mesh sieve was retained for use.

Preparation of Balady bread

The balady bread samples were prepared according to the modified method of Saba, (1985). Formulation of the bread is applied as follow: wheat flour, 1000 g; salt, 20g; and dries yeast, 2 g; and water 500 g. Yeast was mixed with water (25 ^{0}C) to form a suspension, to which the other ingredients were then added and kneaded to form smooth dough. Substitution of wheat flour with potato peel powder (PPP), cauliflower leaves powder (CLP), red onion skin powder (ROSP) and mango peel powder (MPP), were conducted based on 5% of the weight of the wheat flour. The dough was later proofed for 2 hours in a proofer (Bakbar E81, New Zealand), then cut into loafs 120 g prior to baking at 170 ^{0} C for 10 min.

Biological Experiments

Materials

Casein was obtained from Morgan Chemical Co., Cairo, Egypt. The rest of chemicals, reagents and solvents were of analytical grade and purchased from El-Ghomhorya for Drugs, Chemicals and Medical Instruments Trading Co. (Cairo, Egypt).

Animals

Animals used in this study, adult male albino rats (130-150 g per each) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Basal Diet

The basic diet prepared according to the following formula as mentioned by (AIN, 1993) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The diet induced obesity (DIO) prepared according to Research Diets, Inc. NJ, as follow: casein, 80 mesh (23.3%), L-cystine (0.35%), corn starch (8.48%), maltodextrin (11.65%), sucrose (20.14%), soybean oil (2.91%), lard fat (20.69%), mineral mixture (1.17%), dicalcium phosphate (1,52%), calcium carbonate (0.64%), potassium citrate.1 H_2O (1.92%), vitamin mixture (1.17%), choline bitartrate (0.23%). The used vitamins and salt mixtures components were formulated according to Campbell, (1963) and Hegested, (1941), respectively.

Experimental design

All biological experiments performed a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 1996). Rats (n= 42 rats), 160-170g per each, were housed individually in wire cages in a room maintained at 25 ± 2 ^{0}C and kept under normal healthy conditions. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group (Group 1, 6 rats) still fed on basal diet and the other main group (36 rats) was feed with diet-induced obesity (DIO) for 8 weeks which classified into sex sub groups as follow: group (2), fed on DIO as a positive control; group (3), fed on DIO containing 5 % PPP; group (4), fed on DIO containing 5 % CLP; group (5), fed on DIO containing 5 % ROSP, group (6), fed on DIO containing 5 % MPP and group (7): fed on DIO containing 5 % mixture, PPP + CLP+ ROSP + MPP by equal parts. Body weight gain (as percent of initial weight) was assayed every week in rats.

Blood sampling

At the end of experiment period, 8 weeks, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum according to Drury and Wallington, (1980). Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20^{o}C until analysis.

Hematological analysis

Blood lipids profile

Triglycerides (TG), Total cholesterol (TC) and HDL-Cholesterol were determined in serum using specific kits purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were assayed according to the equations of Fniedewald *et al.*, (1972) *as* follow:

Very low density lipoprotein (VLDL cholesterol) = TG/5

LDL cholesterol = Total cholesterol – HDL cholesterol – V LDL cholesterol

Liver functions

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) were determined according to Yound, (1975) and Tietz, (1976) respectively by using specific kits supplied by Biocon Company, Cairo, Egypt.

Kidney functions

Serum creatinine and urea concentration was determined using the modified kinetic methods of **Young *et al.*, (1975)** and **Chaney *et al.*, (1962)** through specific kits supplied by Biocon Company, Cairo, Egypt, respectively.

Serum glucose

Enzymatic determination of serum glucose was carried out colorimetrically according to Yound, (1975).

Albumin

Albumin was determined in plasma using kits purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt.

Protease activity assay

The protease activity was determined by adaptation the method of Rinderrnecitt *et al*., (1968). Briefly, 100 μl of plasma were added to 40 μ l of buffer (150 mM Tris base, 30 mM $CaCl_2$, 0.05% Brij 35) and 50 μl of protease substrate (20% Hide powder azure, HPA, 20 % sucrose, 0.05% Brij). The tubes contents were incubated at $37^{0}C$ with continuous shaking for 2 hours. The reaction was stopped by the addition of 50 μl of 10% TCA and the tubes were stored at $4^{0}C$ for about 15 min. After spined the tubes at 8500 rpm for 5 min, the supernatants were transferred to new tubes and the absorbencies were measured at 540 nm. Blank tubes were prepared by the same previous steps without samples addition.

Statistical Analysis

All measurements were done in triplicate and recorded as mean±SD. Statistical analysis was performed with the Student *t*-test and MINITAB-12 computer program (Minitab Inc., State College, PA).

Results and Discussion

The effect of food processing by-products applied in bread on body weight of obese rats

The effect of food processing by-products applied in bread on body weight (BW, Percent of baseline) of obese rats was shown in Table (1) and Figure (1). From such data it could be noticed that feeding of rats on diet induced obesity (DIO) leads to increase the BW than the control group. At the end of the experiment (8 weeks), rats of the normal group recorded 183.27% of baseline for the BW while obese group was 221.50% of baseline. Replacement of wheat flour with potato peel powder (PPP), cauliflower leaves powder (CLP), red onion skin powder (ROSP), mango peel powder (MPP) and their mixture induced significant decreasing on BW of the obese rats which recorded 205.47, 210.48, 196.91, 198,61 and 191.09 % of baseline, respectively. The higher effect on weigh decreasing was recorded for the by-product mixtures followed by ROSP, MPP, PPP and CLP, respectively. The effect of different plant parts including PPP, CLP, ROSP, and MPP in the control of obesity is the main subjects of many studies (El-Nashar, 2007; Bedawy, 2008; Bonet et al., 2015 and Sayed Ahmed, 2016). The positive effects of such plant parts regarding the control of the obesity could be attributed to their high level content of different classes phytochemical compounds including flavonols, phenolic acids, anthocyanins, alkaloids, carotenoids, phytosterols and organosulfur compounds (Onyeneho and Hettiarachchy, 1993; Rodriguez *et al*., 1994;

Velioglu *et al*., 1998; Beattic *et al*., 2005 and Mashal, 2016). Such bioactive compounds and their conversion products have been shown to impact gene expression and cell (including adipocyte) function through multiple mechanisms, notably by: (a) interacting with several transcription factors of the nuclear receptor superfamily, (b) interfering with the activity of other transcription factors, (c) modulating signaling pathways which are associated with inflammatory and oxidative stress responses; and (d) through extragenomic actions including scavenging of reactive species,

Table 1. The effect of food processing by-products applied in bread on body weight gain (Percent of baseline) of obese rats*

Groups	Feeding period (weeks)								
	0	1	2	3	4	5	6	7	8
Control (-) Std diet	100.00	106.33	115.12	121.94	138.24	158.73	168.72	175.54	183.72
Control (+) Obese	100.00	113.14	128.11	134.60	156.95	192.60	200.02	207.74	221.50
PPP	100.00	110.69	120.44	126.66	146.45	180.62	188.0	193.14	205.47
CLP	100.00	112.29	123.67	129.35	150.83	185.09	192.05	197.25	210.48
ROSP	100.00	105.95	118.71	122.92	143.34	169.68	176.70	184.96	196.91
MPP	100.00	109.62	120.55	127.41	148.57	169.88	178.53	186.82	198.61
PPP + CLP + ROSP + MPP	100.00	108.80	117.66	124.58	141.23	161.38	173.09	180.09	191.09

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts.

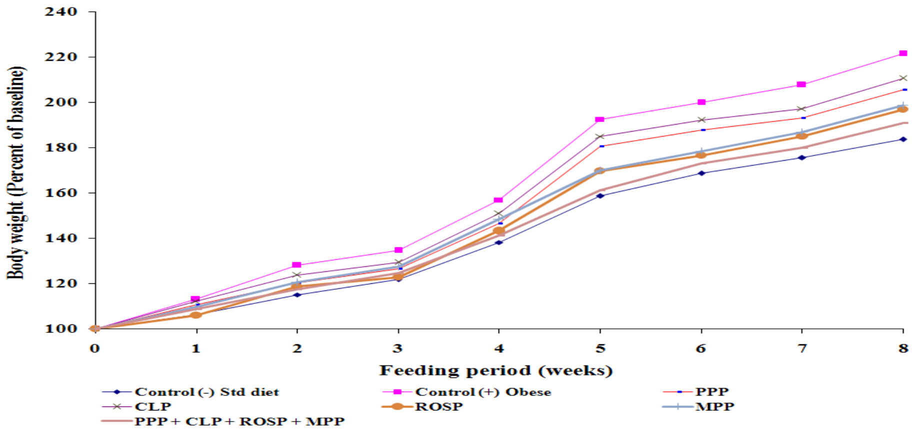

Figure 1. The effect of food processing by-products applied in bread on body weight gain (Percent of baseline) of obese rats*

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts.

retinoylation (Constance *et al.,* 2003; Bonet *et al.,* 2015 and Sayed Ahmed, 2016) . All of these mechanisms contribute to their action control of adipocyte function, adiposity and obesity (reviewed in Bonet *et al.,* 2015).

The effect of food processing by-products applied in bread on blood lipids profile of obese rats

The effect of food-processing by-products applied in bread on some blood lipid profile parameters in plasma of obese rats were shown in Table (2) and Figure (2). From such data it could be noticed that obesity induced a significant increased ($p \leq 0.05$) in TG (32.52%), TC (23.04) and LDL (51.78%) while significant decreased ($p \leq 0.05$) in HDL (29.85%) compared to normal controls. Replacement of wheat flour with PPP, CLP, ROSP, MPP and their mixture induced significant improvements on blood lipid profile through decreasing the TG, TC and LDL by the ratio of 16.06, 22.97, 11.70, 13.45 and 5.86; 16.06, 22.97, 11.70, 13.45 and 5.86; and 32.15, 36.33, 24.16, 30.20 and 17.47%, respectively. The opposite direction was observed for the HDL levels. The higher effects in improving of the blood lipid profile disorders induced by obesity in rats were recorded for the by-product mixtures followed by ROSP, MPP, PPP and CLP, respectively. In the same context, modeling based on systematic reviews of RCTs suggests that modest and sustained weight loss (5-10 kg) in patients with overweight or obesity is associated with reductions in low density lipoprotein, total cholesterol and triglycerides and with increased levels of high density lipoprotein. (Stenius-Aarniala *et al.,* 2000; Neter *et al,* 2003; Avenell *et al.,* 2004; Poobalan *et al.,* 2004; Christensen *et al.,* 2007; Bales and Buhr, 2008 and Williamson *et al.,* 2009).

In this context, coronary heart disease (CHD) is a major health problem in both industrial and developing countries including Egypt. Many studies have now shown that blood elevated concentrations of total or low density lipoprotein (LDL) cholesterol in the blood are powerful risk factors for CHD, whereas high concentrations of high density lipoprotein (HDL) cholesterol or a low LDL (or total) to HDL (reviewed in Bedawy, 2008). The composition of the human diet plays an important role in the management of lipid and lipoprotein concentrations in the blood. Reduction in saturated fat and cholesterol intake has traditionally been the first goal of dietary therapy in lowering the risk for cardiovascular disease. In recent years, however, the possible hypocholesrerolemic effects

Table 2. The effect of food processing by-products applied in bread on blood lipids profile of obese rats*

Value	Control (-)	Control (+)	Food processing by-products (5%, w/w)				
			PPP	CLP	ROSP	MPP	Mixture
Triglycerides (TG, mg/dL)							
Mean	81.77[d]	108.36[a]	94.9 [c]	100.55[b]	91.34[c]	92.77[c]	86.56[d]
SD	2.33	4.23	2.87	5.98	3.88	5.01	2.39
% of Change	0.00	32.52	16.06	22.97	11.70	13.45	5.86
Total cholesterol (TC, mg/dL)							
Mean	139.13[e]	171.19[a]	157.61[bc]	160.88[b]	153.78[c]	156.49[c]	149.87[d]
SD	5.98	7.21	7.61	8.44	4.12	9.88	8.11
% of Change	0.00	23.04	13.28	15.63	10.53	12.48	7.72
High density lipoprotein (HDL, mg/dL)							
Mean	45.12[a]	31.65[c]	36.01[b]	34.90[b]	39.09[a]	36.83[b]	41.34[a]
SD	2.11	3.12	1.99	4.33	2.89	4.03	2.18
% of Change	0.00	-29.85	-20.19	-22.65	-13.36	-18.37	-8.38
Low density lipoprotein (LDL, mg/dL)							
Mean	77.66[e]	117.87[a]	102.62[b]	105.87[b]	96.42[c]	101.11[b]	91.22[d]
SD	1.98	3.21	7.61	3.44	2.12	8.88	5.11
% of Change	0.00	51.78	32.15	36.33	24.16	30.20	17.47

*PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts. Means in the same row with different litters are significantly different at $p < 0.05$.

of several dietary components, such as found in our selected plant parts (PPP, CLP, ROSP and MPP) including, flavonols, phenolic acids, anthocyanins, alkaloids, carotenoids, phytosterols and organosulfur compounds etc have attracted much interest. Also, phenolic compounds found in such plant parts exerts its beneficial effects on cardiovascular health by antioxidant and anti-inflammatory activities *(*Anonymous, 1998 and Kuhlmann *et al., 1998).* LDL oxidation and endothelial cell damage is believed to be involved in the early development of atherosclerosis *(*Kaneko *et al., 1994)*. Researchers found that presence of phenolics such quercetin significantly reduced LDL oxidation *in vitro* from various oxidases including 15-lipoxygenase, copper-ion, UV light, and linoleic acid hydroperoxide *(*Aviram *et al.*, 1999 and Kaneko *et al., 1994).*

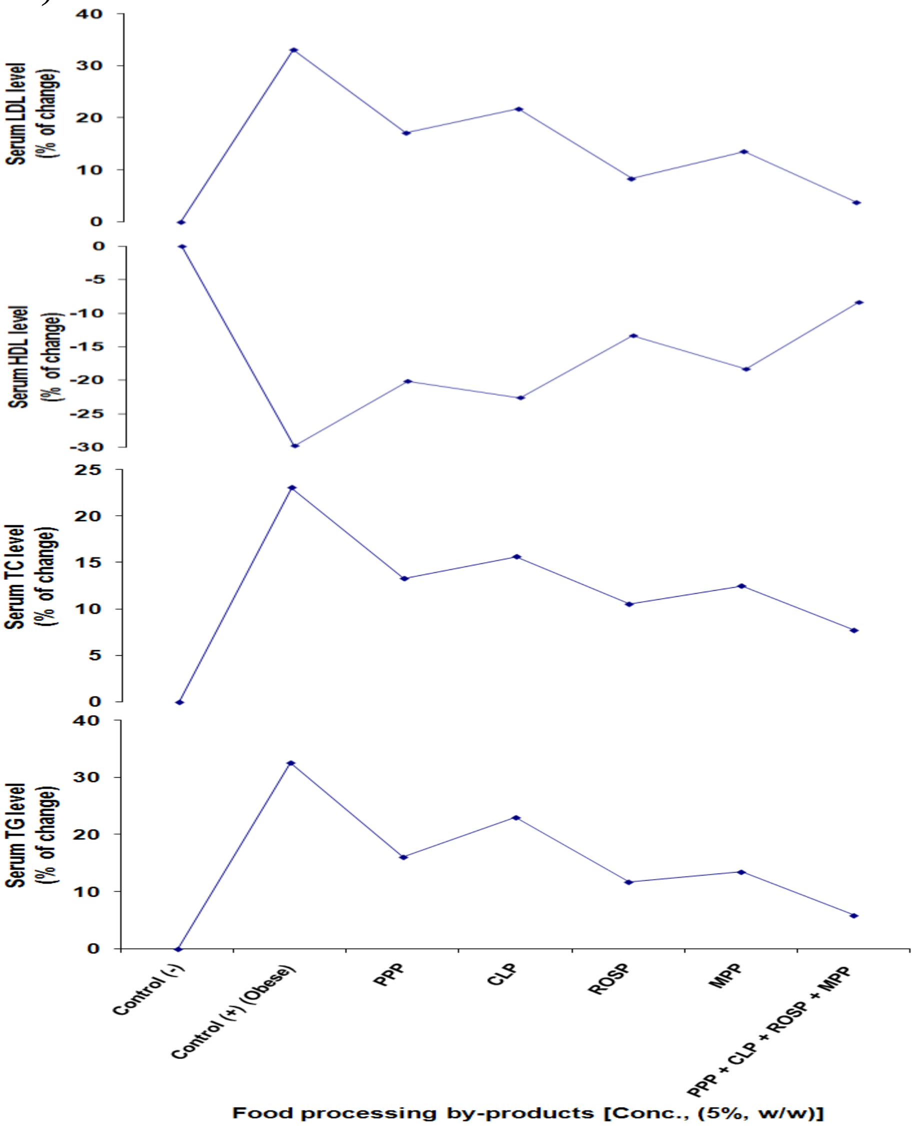

Figure 2. The effect of food processing by-products applied in bread on blood lipids profile (as a % of change) of obese rats*

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts.

Besides the direct antioxidant effect, quercetin also inhibited consumption of *alpha*-tocopherol *(*Hertog *et al.,* 1992 and Kaneko *et al., 1994)* and protected human serum paraxonase (PON 1) activities *(*Aviram *et al., 1999).* Also, McAnlis *et al., 1999)* suggested that quercetin, having a high affinity for protein, was bound to albumin and never incorporated into the LDL particle.

The effect of food-processing by-products applied in bread on liver functions enzymes activity in plasma of obese rats

The effect of food-processing by-products applied in bread on liver function enzymes activity in plasma of obese rats were shown in Table (3) and Figure (3). From such data it could be noticed that obesity induced a significant increased ($p \leq 0.05$) in ALT (21.80%) and AST (42.82.55%) compared to normal controls. Replacement of wheat flour with potato peel powder (PPP), cauliflower leaves powder (CLP), red onion skin powder (ROSP), mango peel powder (MPP) and their mixture induced significant decreasing on liver ALT and AST activities by the ratio of 4.66, 12.15, 2.13, 2.36 and 0.94; and 21.45, 28.49, 11.64, 20.07 and 8.50%, respectively. The higher effects in manipulation of the liver enzymes disorders induced by obesity in rats were recorded for the by-product mixtures followed by ROSP, MPP, PPP and CLP, respectively.

Aminotransferases are normally intracellular enzymes. Thus, the presence of elevated levels of aminotransferase in the plasma indicates damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lysis, resulting release of intracellular enzymes into the blood. Two amino transferases were found in plasma are of particular diagnostic value AST and ALT. AST enzyme is one of the enzymes tested in the cardiac enzyme series. This enzyme is found in very high concentration within the heart muscles, skeletal muscle cells, and to a leaser degree in the kidney and panaceas. ALT is found predominately in the liver leaser quantities are found in the kidneys, heart and skeletal muscles (Pagana and pagana, 1997). These enzymes are elevated in nearly all liver diseases, but are particularly high in conditions that the causes extensive cell necrosis, such as severe viral hepatitis and prolongated circulatory collapse. Serial enzyme measurments are often useful in determining the course of liver damage (Abd El-Aziz, 1990; Pagana and pagana, 1997 and Hong *et al.,* 2002).

Also, aminotransferases may be elevated in nonhepatic disease, such as myocardial infraction and

Table 3. The effect of food processing by-products applied in bread on liver functions of obese rats*

Value	Control (-)	Control (+)	Food processing by-products (5%, w/w)				
			PPP	CLP	ROSP	MPP	Mixture
Serum alanine amino transferase activity (ALT, U/L)							
Mean	47.88 *ab	58.32 a	50.11 ab	53.65 ab	48.9 ab	49.01 ab	48.33 ab
SD	5.8	4.67	6.09	4.87	3.76	6.91	4.45
% of Change	0.00	21.80	4.66	12.05	2.13	2.36	0.94
Serum aspartate amino transferase activity (AST, U/L)							
Mean	26.11 c	37.29 a	31.71 ab	33.55 ab	29.15 ab	31.35 ab	28.33 abc
SD	4.33	5.02	3.78	3.66	3.02	2.11	3.11
% of Change	0.00	42.82	21.45	28.49	11.64	20.07	8.50

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts. Means in the same row with different litters are significantly different at $p < 0.05$.

muscle disorders; however, these disorders can usually be distingushed clinically from liver disease (Champe and Harvey, 1994). Data of the present study with the other reported that aminotransferases may be elevated significantly in additionally nonhepatic disease such as obesity in human and experimental animals (Elhassaneen and Salem, 2014).

Such as reviewed in many studies plant parts including PPP, CLP, ROSP, and MPP are a rich source of different classes of phytochemicals such flavonols, phenolic acids, anthocyanins, alkaloids, carotenoids, phytosterols and organosulfur compounds (Harborne, 1998; Onyeneho and Hettiarachchy, 1993; Rodriguez *et al*., 1994; Velioglu *et al*., 1998; Singh *et al.,* 2002; Beattic *et al.,* 2005; Mohamed, 2012 and Mashal, 2016). The present study with others reported that the effect of many plant parts on decreasing the serum liver function enzymes activity could be attributed to their high level content of that phytochemicals (Hassan, 2011; Abd El-Fatah, 2013 and Elhassaneen *et al.,* 2013). The possible mode of action of liver serum enzymes-lowering activity of the tested bread supplemented with by-products including ROSP, MPP, PPP and CLP, as individually or mixture, could be explained by one or more of the following process. Flavonoids found in all the tested by-products are known to block the hepatocellular uptake of bile acids (Dawson, (1998). Flavonoids pretreatment improved the antioxidant capacity of the

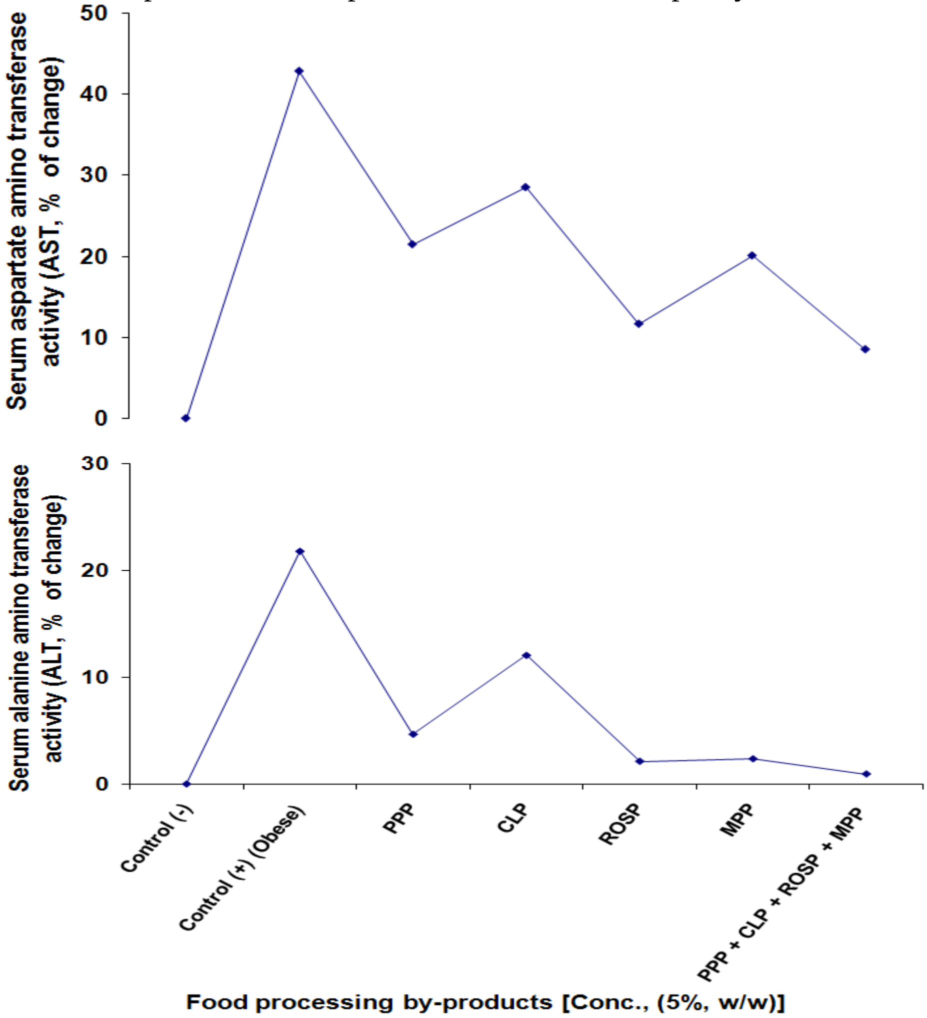

Figure 3. The effect of food processing by-products applied in bread on liver functions of obese rats*

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts

liver, diminished the bilirubin concentration compared with the groups without treatment (Beattic *et al.*, 2005). They also reported that flavonol glycosides reduced the elevated levels of the following serum enzymes, AST, ALT and ALP. Als, pre-treatment with flavonoids were not only able to suppress the elevation of AST and ALT but also reduce the damage of hepatocytes *in vitro* was reported by El-Nashar, (2007). It was found that flavonoids have exhibited strong antioxidant activity against reactive oxygen species (ROS) *in vitro*. The hepatoprotective activity of flavonoids was possibly due to its antioxidant properties, acting as scavengers of reactive oxygen species (ROS). Pre-treatment with apricot kernel extract rich in phytochemicals were able to reduce the damage of liver i.e. suppresses the elevation of AST, ALT and ALP through the improvement of antioxidant defense system in red blood cells (Hassan, 2011). Take in our consideration all of these mechanisms of actions, the higher improvement in liver function parameters recorded in rats feeding food processing by-products mixture bread samples could be attributed to the antagonism effects induced by their content of different phytochemical catagories.

Effect of plant by-products applied in bread on kidney functions in plasma of obese rats

Kidney functions (urea and creatinine concentrations) in serum of obese rats consumed food processing by-products applied in bread were shown in Table (4) and Figure (4). From such data it could be noticed that obesity induced a significant increased ($p \leq 0.05$) in urea (17.12%) and creatinine (21.57%) compared to normal controls. Supplementation of the rat diets with 5% w/w by PPP, CLP, ROSP, MPP and their mixture induced significant decreasing on serum urea and creatinine levels by the ratio of 8.23, 10.32, 5.03, 7.06 and 3.18; and 13.73, 15.69, 3.92, 9.80 and 5.88%, respectively. The higher amelioration effects in kidney disorders induced by obesity in rats were recorded for the by-product mixtures treatment followed by ROSP, MPP, PPP and CLP, respectively.

In general, urea is formed in the liver as the end product of protein metabolism. During ingestion, protein is broke down into amino acids. In the liver, these amino acids are catbolized and free ammonia is formed. The ammonia is combined to form urea (Pagana and pagana, 1997). Urea,

Table 4. The effect of food processing by-products applied in bread on kidney functions of obese rats*

Value	Control (-)	Control (+)	Food processing by-products (5%, w/w)				
			PPP	CLP	ROSP	MPP	Mixture
Serum urea concentration (mg/dl)							
Mean	48.14 [*ab]	56.38 [a]	52.1 [ab]	53.11 [ab]	50.56 [ab]	51.54 [ab]	49.67 [ab]
SD	2.76	4.11	4.34	3.11	2.56	5.14	3.56
% of Change	0.00	17.12	8.23	10.32	5.03	7.06	3.18
Serum creatinine concentration (g/dl)							
Mean	0.51 [ab]	0.62 [a]	0.58 [a]	0.59 [a]	0.53 [ab]	0.56 [a]	0.54 [ab]
SD	0.04	0.08	0.11	0.06	0.04	0.07	0.1
% of Change	0.00	21.57	13.73	15.69	3.92	9.80	5.88

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts. Means in the same row with different litters are significantly different at $p < 0.05$.

the major product of protein catabolism measuring urea is the most popular laboratory procedure for assessing renal function (Bennett *et al*., 1995 and Pagana and pagana, 1997). Creatinine is a catabolic product of creatine phosphate, which is used in skeletal muscle concentration (Pagana and pagana, 1997). In the skeletal muscle serum creatinine levels are elevated by renal disease and dehydration. The decreasing in serum uric acid and creatinine as the result of feeding plant parts including PPP, CLP, ROSP and MPP could be attributed to their higher content of phytochemicals such flavonols, phenolic acids, anthocyanins, alkaloids, carotenoids, phytosterols and organosulfur compounds (Harborne, 1998; Rodriguez *et al*., 1994; Velioglu *et al*., 1998; Singh *et al.,* 2002; Beattic *et al.,* 2005; Mohamed, 2012 and Elhassaneen *et al.,* 2013). Different doses of such bioactive compounds found in cinnamon extract showed slight-decreased in serum creatinine after 12 week of feeding when compared with control group (El-Nashar, 2007). The decreasing in serum uric acid and creatinine as the result of feeding onion, garlic and cabbage could be attributed to their higher content of phenolic compounds (Bedawy, 2008). Also, active ingredients in sweet violet (*Viola odorata* L.) blossom powder prevented partially the rise of mean serum urea and creatinine levels induced by CCl_4 injection (Abd El-Fatah, 2013 and Elhassaneen *et al.,* 2013).

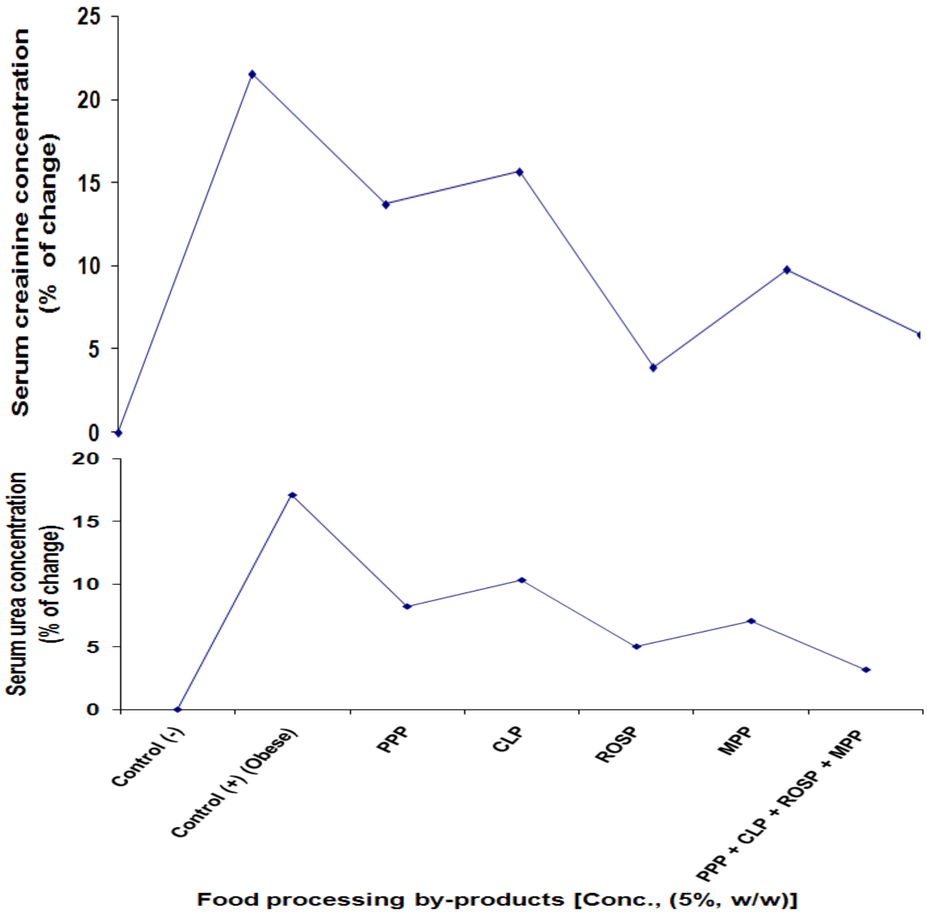

Figure 4. The effect of food processing by-products applied in bread on kidney functions of obese rats*

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts.

The possible mode of action of kidney serum parameters-lowering level of the tested by-products could be explained by one or more of the following process. Polyphenols found in such plant by-products improved the kidney weight and serum levels of urea nitrogen, creatinine and creatinine clearance as well as increased the activity of superoxide dismutase in the kidney (reviewed in El-Nashar, 2007). Also, flavanone produced significant protection of renal function by significant reduction in serum urea and creatinine concentrations, decreased polyuria and reduction in body weight loss, marked reduction in urinary fractional sodium excretion as well as protected kidney tissues (Badary et al., 2005 and Mohamed et al., 2005). Finally, flavonoids lowered plasma creatinine and urea concentration, both indicating a better postoperative kidney functions (Sayed Ahmed, 2016). Such mechanisms of action indicated that a combination of different plant by-products may be more efficient for reducing serum level of urea and creatine, the biomarkers of kidney functions stress, because the interactive effects occurred by different categories of bioactive compounds of plant by-products used.

Effect of food processing by-products applied in bread on serum glucose of obese rats

Glucose concentration in serum of obese rats consumed food processing by-products applied in bread was shown in Table (5) and Figure (5). From such data it could be noticed that obesity induced a significant increased ($p \leq 0.05$) in serum glucose (38.63%) compared to normal controls. Supplementation of the rat's diets with 5% w/w by PPP, CLP, ROSP, MPP and their mixture induced significant decreasing on serum glucose concentrations by the ratio of 21.81, 24.32, 10.51, 16.27 and 7.23%, respectively. The higher amelioration effect in serum glucose rising induced by obesity in rats was recorded for the by-product mixtures treatment followed by ROSP, MPP, PPP and CLP, respectively.

In similar studies, in patients with type 2 diabetes, weight loss of around 5 kg is associated with a reduction in fasting blood glucose of between 0.17 mmol/L to 0.24 mmol/L at 12 months (Avenell *et al.,* 2004 and Vettor *et al.,* 2005). The decreasing in serum glucose as the result of feeding plant parts and by-products including PPP, CLP, ROSP and MPP was the subject of many studies. For example, significant research has

Table 5. The effect of food processing by-products applied in bread on serum glucose concentration (mg/dL) of obese rats*

Value	Control (-)	Control (+)	Food processing by-products (5%, w/w)				
			PPP	CLP	ROSP	MPP	Mixture
Mean	98.23*e	136.18a	119.65 b	122.12 b	108.55d	114.21cd	105.33d
SD	5.21	6.11	3.32	6.31	5.89	6.43	4.15
% of Change	0.00	38.63	21.81	24.32	10.51	16.27	7.23

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts. Means in the same row with different litters are significantly different at p < 0.05.

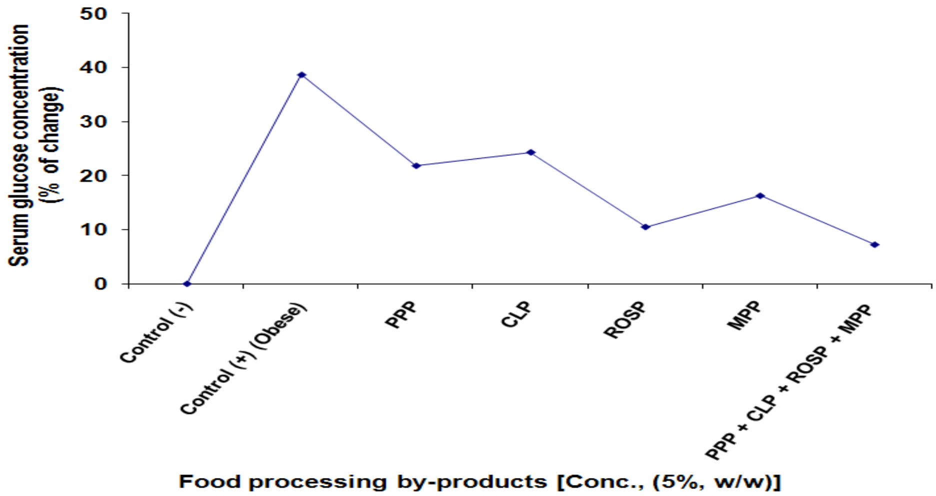

Figure 5. The effect of food processing by-products applied in bread on serum glucose concentration (as a % of Change) of obese rats*

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts.

been done on the effect of onion consumption on diabetic conditions. The organosulfur compounds S-methylcysteine sulfoxide and S-allylcysteine sulfoxide were linked to significant amelioration of weight loss, hyperglycemia, low liver protein and glycogen, and other characteristics of diabetes mellitus in rats (Sheela *et al.*, 1995). Onion peel extract (OPE) might improve glucose response and insulin resistance associated with type 2 diabetes by alleviating metabolic dysregulation of free fatty acids, suppressing oxidative stress, up-regulating glucose uptake at peripheral tissues, and/or down-regulating inflammatory gene expression in liver which was reported by Jung *et al.*, (2011). Moreover, in most cases, OPE showed greater potency than pure quercetin equivalent. These findings provide a basis for the use of onion peel to improve insulin insensitivity in type 2 diabetes. OPE might improve glucose response and insulin resistance associated with type 2 diabetes by alleviating metabolic dysregulation of free fatty acids, suppressing oxidative stress, up-regulating glucose uptake at peripheral tissues, and/or down-regulating inflammatory gene expression in liver. Moreover, in most cases, OPE showed greater potency than pure quercetin equivalent. These findings provide a basis for the use of onion peel to improve insulin insensitivity in type 2 diabetes. PPP and pomegranate peel powder (PGPP) displays potent hypoglycemic action in alloxane-induced diabetic rats. Such activity may be related to diverse phenolic compounds present in PGPP and PPP including punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3,5-diglucosides) chlorogenic, gallic, protocatechuic and caffeic acids (Onyeneho and Hettiarachchy, 1993 and Rodriguez *et al*., 1994). These compounds are known for their properties in scavenging free radicals, inhibiting lipid oxidation *in vitro* and improve glucose response and insulin resistance associated with type 2 diabetes (Gil *et al.*, 2000; Noda *et al.*, 2002 and Jung *et al.*, (2011). Additionally, the mixture treatment gave maximum hypoglycemic yield when compared with the tested by-products separated. It could be mean that a combination of different by-products may be more efficient for reducing the serum glucose level because the interactive effects occurred by different categories of bioactive compounds of plant parts used.

Effect of food processing by-products applied in bread on immunological parameters of obese rats

Some immunological parameters (albumin level and protease activity) in serum of obese rats consumed food processing by-products applied in bread were shown in Table (6) and Figure (6). From such data it could be noticed that obesity induced a significant decreased ($p \leq 0.05$) in albumin level (15.09%) and protease activity (12.85%) compared to normal controls. Supplementation of the rat diets with 5% w/w by PPP, CLP, ROSP, MPP and their mixture induced significant increasing on albumin level and protease activity by the ratio of 812.05, 13.01, 8.88, 11.29 and 6.21; and 9.03, 10.07, 7.99, 8.68 and 6.25%, respectively. The higher amelioration effects in immunological disorders induced by obesity in rats were recorded for the by-product mixtures treatment followed by ROSP, MPP, PPP and CLP, respectively.

Albumin is an important metal binding protein. It is a sacrificial antioxidant that can bind copper tightly and iron weakly to its surface serving as a target for their related free radical reactions. Thus it inhibits copper ion dependent lipid peroxidation (Gutteridge and Wilkins, 1983). It was reported that hypo-albuminaemia is most frequent in the presence of advanced chronic liver diseases. Hence decline in total protein and albumin can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. So, it is worthy to report that feeding with some food industry by-produts including ROSP, MPP, PPP and CLP produced significant improvement in serum albumin compared to obese group. Such findings are in accordance with that observed by Ahmed, (2014) who found that pretreatment with cape gooseberry fruits extract produced significant improvement in serum total protein, albumin, and total globulin compared to cisplatin (kidney failure inducer) group.

On the other side, numerous studies have revealed that proteases could play an important role in immunological functions and humoral host defense (reviewed in Neurath ,1989; Troll and Kennedy, 1993). Other studies outlining the direct relationship between protease expression and protozoal virulence have implicated proteases as being involved in pathogenicity. In several studies, protease activity was found decreased as found in the present study. For example, Elhassaneen *et al*., (1997) and Elhassaneen, (2001) reported that a significant decreasing in the protease activity of

Table 6. The effect of food processing by-products applied in bread on immunological parameters of obese rats*

Value	Control (-)	Control (+)	Food processing by-products (5%, w/w)				
			PPP	CLP	ROSP	MPP	Mixture
Serum albumin level (Alb, g/L)							
Mean	48.97[*a]	41.58 [abc]	43.07[abc]	42.60[abc]	44.62[ab]	43.44[abc]	45.93[ab]
SD	4.1	2.67	4.33	2.98	1.59	4.14	3.1
% of Change	0.00	-15.09	-12.05	-13.01	-8.88	-11.29	-6.21
Serum protease activity (PA, U/L)							
Mean	2.88[a]	2.51[ab]	2.62 [ab]	2.59 [ab]	2.65 [ab]	2.63 [ab]	2.70 [a]
SD	0.41	0.3	0.52	0.11	0.34	0.4	0.16
% of Change	0.00	-12.85	-9.03	-10.07	-7.99	-8.68	-6.25

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts. Means in the same row with different litters are significantly different at $p < 0.05$.

fish isolated liver cells as a consequence of exposure to paper industry effluent, pesticides, heavy metals, polycyclic aromatic hydrocarbons etc. Co-treatment of liver cell with CCl_4 and the tested selected plant parts extracts as well as their mixture exhibited therapeutic effects through decreasing the immunotoxic effects i.e. protease activity.

Research has examined a variety of natural substances such as found the the present selected by-products i.e. ROSP, MPP, PPP and CLP that demonstrate immunomodulatory potential.

Immunomodulation is described as the ability of a nutrient, herb, or other substance to promote healthy immune function (Brown, 1996). Certain plant compounds have been shown in experimental studies to have immunostimulating properties; that is, they appear to help stimulate viral defense mechanisms by activating immune cells such as macrophages, lymphocytes (T and B-cell, and natural killer cell), and the cytokines (e.g., interleukin, interferon, and tumor necrosis factor) (Roesler, 1991 and Suresh and Vasudevan, 1994). That decreasing in immunotoxic effects was depending on the type of the plant parts applied. The highest therapeutic effect was recorded for the mixture of the selected plant parts which could be attributed to the antagonism effects as the result of different phytochemicals categories including (Schieber *et al.*, 2001; Sayed Ahmed, 2016).

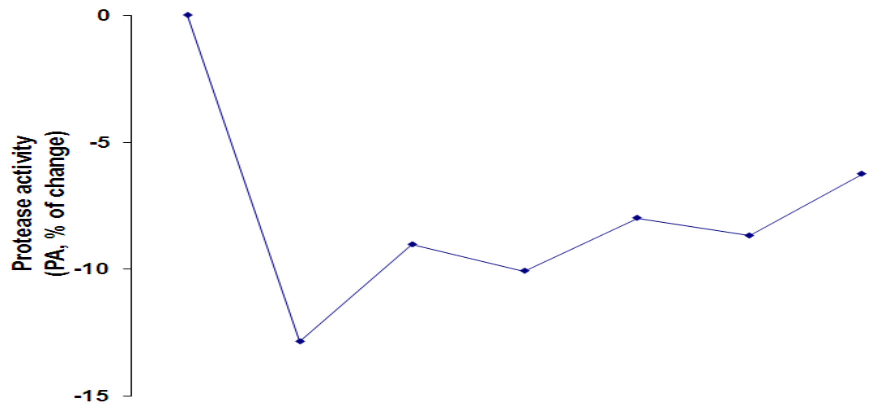

Figure 6. The effect of food processing by-products applied in bread on immunological parameters of obese rats.*

* PPP, potato peel powder, CLP; cauliflower leaves powder; ROSP, red onion skin powder; MPP, mango peel powder and Mixture, PPP + CLP+ ROSP + MPP by equal parts.

In conclusion, the present study has demonstrated the potency of the selected food processing by-products including PPP, CLP, ROSP and MPP to ameliorate liver, kidney, blood lipids profiles disorders and hyperglycemia in obese rats. Furthermore, PPP, CLP, ROSP and MPP improved the immunological functions i.e. increasing the albumin level and protease activity in serum. These findings provide a basis for the use of PPP, CLP, ROSP and MPP and also have important implications for the prevention and early treatment of obesity. Also, more research must be done on the future to elucidate the realized benefits from dietary food processing by-products intake on obesity disease and its complications.

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التغذية على النواتج الثانوية للصناعات الغذائية لتحسين مضاعفات مرض السمنة في الفئران

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ملخص البحث :
تهدف الدراسة إلى التعرف على تاثير خلط النواتج الثانوية للصناعات الغذائية بالخبز البلدي في تحسين مضاعفات السمنة في الفئران. تم تقسيم اثني وأربعون فأر (١٦٠-١٧٠جم) إلى مجموعتين رئيسيتين، المجموعة الأولى (مجموعة ١، ٦ فئران) تم تغذيتها على الغذاء الأساسي (مجموعة ضابطة سالبة) والمجموعة الرئيسية الأخرى (٣٦ فار) تم تغذيتها علي نظام غذائي يسبب السمنة (DIO) لمدة ٨ أسابيع، تم تقسيمها فيما بعد إلى ستة مجموعات فرعية على النحو التالي: المجموعة (٢) تم تغذيتها على غذاء (DIO) كمجموعة ضابطة موجبة، اما المجموعات (٣ - ٧) تم تغذيتها علي غذاء DIO يحتوي على ٥٪ من مسحوق قشر البطاطس (PPP)، مسحوق اوراق القنبيط (CLP)، مسحوق قشر البصل الاحمر (ROSP)، مسحوق قشر المانجو (MPP) ومسحوق مخلوطهم، على التوالي. تغذية الفئران على نظام غذائي يسبب السمنة لمدة ثمانية أسابيع ادي إلى زيادة وزن الجسم عند مقارنتها بالمجموعة الضابطة وقد سجلا ١٨٣.٢٧، ٢٢١.٥٠%، علي التوالي. بينما استبدال دقيق القمح بنسبة ٥٪ من PPP، CLP، ROSP، MPP ومخلوطهم ادي الي انخفاض كبير في اوزان الفئران التي تعاني السمنة وقد سجلت ٢٠٥.٤٧، ٢١٠.٤٨، ١٩٦.٩١، ١٩٨.٦١ و ١٩١.٠٩٪ ، على التوالي. أشارت النتائج أن السمنة ادت الي زيادة معنوية في كل من نسبة الدهون في الدم (TG، ٣٢.٥٢٪، TC، ٢٣.٠٤٪ و LDL، ٥١.٧٨٪)، وظائف الكبد (ALT، AST ٣٣.٦٥٪ و، ٣٢.٥٤٪)، وظائف الكلى (حامض البوليك، ٣٣.٦٨٪، والكرياتينين، ٢٩.٦٢٪) والجلوكوز في مصل الدم (٣٨.٦٣٪) مقارنة بالمجموعة الضابطة السالبة، كما اظهرت بعض التقديرات المناعية (مستوى الالبيومين ونشاط الأنزيم البروتيني) في مصل الدم لدي الفئران التي تعاني من السمنة وجود انخفاض معنوي ($p\leq0.05$). وجد ان التغذية على ٥٪ من PPP، CLP، ROSP، MPP ومسحوق مخلوطهم ادي الي تحسن ملحوظ في كل التقديرات السابقة. وقد سجلت افضل التأثيرات لمخلوط النواتج ثم مسحوق قشر البصل الاحمر، مسحوق قشر المانجو، مسحوق قشر البطاطس و مسحوق اوراق القنبيط، علي التوالي. استنتجت الدراسة ان النواتج الثانوية للصناعات الغذائية لها انعكاسات مهمة للوقاية والعلاج المبكر من مضاعفات السمنة.

كلمات مفتاحية: قشر البطاطس – اوراق القنبيط – قشور البصل – قشور المانجو – وظائف الكبد – وظائف الكلي –دهنيات الدم – مستوي الجلوكوز.