The effect of Plant by-products on liver functions and plasma glucose in rats fed a high fat diet induced obesity.

تأثير نواتج النباتات الثانوية علي وظائف الكبد ومستوي السكر في الدم للفئران التي تم تغذيتها علي نظام غذائي عالي الدهون مسبب للسمنه.

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Abstract

The present study examined the effects of dietary supplementation of four plant by-products (potato peel, cauliflower leaves, onion skin, and mango peel) in therapeutic nutrition through evaluating the effectiveness of these plant by-products mixed in bread in modulating obesity parameters using obese rat model.

All (35) adult male rats, 150 ±10g, 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(-) , 5 rats still fed on basal diet and the other main group (30 rats) was feed with diet-induced obesity (DIO) for 4 weeks which classified into sex sub groups as follow: Group (+): fed on DIO as a positive control Group (PPP), fed on DIO containing 5 % PPP, Group (CLP), fed on DIO containing 5 % CLP ,Group (ROSP), fed on DIO containing 5 % ROSP, Group (MPP), fed on DIO containing 5 % MPP, Group (Mixture): fed on DIO containing 5 % mixture, PPP + CLP+ ROSP + MPP by equal parts.

At the end of the experimental period (4 weeks) blood was collected then glucose and liver and functions were determined in the serum. The results indicated that supplementation of the rat diets with 5% w/w by PPP, CLP, ROSP, MPP and their mixture induced significant decreasing on serum glucose concentrations liver AST, ALT and ALP activities.

*** 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.**

الملخص

توضح الدراسة أثر استخدام النواتج الثانوية النباتية (قشر البطاطس وأوراق القرنبيط، وقشر البصل، وقشر المانجو) في القيمة الغذائية العلاجية عن طريق تقييم مدي فاعلية إضافة مساحيق من تلك النواتج مع الخبز في الحد من السمنة وفحص اثر تلك النواتج علي وظائف الكبد ومستوي الجلوكوز في الدم لفهم مدي تأثيرها علي امراض الكبد والسكر المرتبطة بالسمنة وذلك عن طريق استخدام فئران مصابة بالسمنة.

تم وضع٣٥ فأر ذكر يزن كل واحد منهم ١٥٠±١٠ جم ، في غرفه درجه حرارتها ٢٥ درجه سليزيه مع مراعاه الظروف الصحيه ، وقد تم تغذيه الفئران علي نظام غذائي ضابط لمده اسبوع قبل اجراء التجربه ثم تم تقسيم الفئران إلى مجموعتين رئيسيتين، المجموعة الأولى: المجموعة الضابطة السالبة (−)التي تتكون من (٥) فئران والتي لا تزال تتغذى على النظام الغذائي الأساسي فقط والمجموعة الثانية (٣٠ فأر) وتتغذي علي نظام غذائي مسبب للسمنة لمدة ٤ أسابيع وتم تقسيمها إلى (٥) مجموعات فرعية على النحو التالي: (١) مجموعة ضابطة ايجابية (+): تتغذى على نظام غذائي مسبب للسمنة ،(٢) مجموعة (مسحوق قشر البطاطس) تم تغذيتها علي نظام مسبب للسمنه تحتوي علي ٥% من مسحوق قشر البطاطس ، (٣) مجموعة (مسحوق ورق القرنبيط) تم تغذيتها علي نظام مسبب للسمنه يحتوي علي ٥% من مسحوق ورق القرنبيط ، (٤) مجموعة (مسحوق قشر البصل) تم تغذيتها علي نظام مسبب للسمنه يحتوي علي ٥% من مسحوق قشر البصل الأحمر ، (٥) مجموعة (مسحوق قشر المانجو) تم تغذيتها علي نظام مسبب للسمنه يحتوي علي ٥% من مسحوق قشر المانجو ، و(٦) مجموعة (مسحوق خليط النواتج النباتية الاربعة) تم تغذيتها علي نظام مسبب للسمنه يحتوي علي ٥% من خليط مكون من (مسحوق قشر البطاطس و مسحوق قشر البصل الاحمر و مسحوق ورق القرنبيط و مسحوق قشر المانجو) بكميات متساوية . وفي نهاية التجربة تم تجميع عينات الدم من الفئران وفصل السيرم لتحليل مستوي الجلوكوز ووظائف الكبد .

أظهرت النتائج أن الفئران المصابة بالسمنه لديها إرتفاع في نسبة السكر في الدم و خلل في انشطه انزيمات الكبد مقارنه بالضوابط الطبيعيه وقد أدي إضافة مساحيق النواتج الثانوية إلي النظام الغذائي للفئران الي إنخفاض ملحوظ في معدلاتها وقد سجل إضافة خليط النواتج الثانوية أعلي نسبة تحسن بالنسبة لزيادتها التي تسببت بها الإصابة بالسمنه .

1. Introduction:

Obesity no longer refers only to being overweight. The World Health Organization (WHO) has officially recognized obesity as a chronic disease and it is defined as an accumulation of adipose tissue that is of sufficient magnitude to impair health (WHO, 2006).The fundamental cause of obesity is an energy imbalance between energy input and output. Generally, this may be due to the increased intake of energy-dense foods and decreased physical activity (Kim, et al., 2015).Obesity is linked to health risks and can lead to various metabolic disorders, such as diabetes, hypertension, and cardiovascular diseases in addition to chronic diseases such as stroke, osteoarthritis, sleep apnea, some cancers, and inflammation-bases pathologies (Singla, et al., 2010; Piper, 2011).

Global strategies are focused on dietary and lifestyle modifi- cations, i.e. restrict caloric intake and increase physical activity to slow obesity development (WHO; 2007). A food field research that has recently aroused considerable interest is the potential of natural products to counteract obesity (Santos et al., 2008 and Park& Kim, 2011). These products contain dietary phytochemical with high potential for health promotion and disease prevention (Visioli et al., 2006). Phytochemicals are defined as bioactive non nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases. (Liu, 2003**).**

The use of these wastes as by-products for further exploitation on the production of food additives or supplements with high nutritional value have gained increasing interest because these are high-value products and their recovery may be economically attractive (Ahmed, 2015). A number of experiments indicate that by-products added to laboratory animals' diet had positive effects on serum lipid profile, liver and kidney functions and serum glucose (Taing, *et al.*, 2012 and Matsunaga *et al.*, 2014). This paper examines the effects of these four plant by-products (potato peel, cauliflower leaves, onion skin, and mango peel) on liver functions and plasma glucose in rats fed a high fat diet induced obesity.

2. Materials and Methods

2.1. Materials

Wheatflour: Variety Giza 155 wheat (*Triticum vulgare*) was obtained from Shebin ElKom market, Menoufiya Governorate, Egypt 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.Salt, bicarbonate soda, yeast and shortening: Were purchased from the local markets of Shebin El-Kom City, Egypt.

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 peel (*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 Shebin ElKom market, Menoufiya Governorate, Egypt during the 2014 harvesting period. The collected samples was transported to the laboratory and used immediately for cauliflower peels preparation.

-Diet ingredients: Casein, cellulose, vitamins and minerals were 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).

- kits: were purchased from Gama Trade Company for chemicals, Cairo.

-Experimental animals (Rats): 35 adult male albino rats (150±10) were used in this study, were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

2.2. Methods:

2.2.1. Preparation of food by-products peel powder:

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.

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.

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.

2.2.2 - Preparation of Balady bread:

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.

2.2.3 - 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=35 rats), 150 ±10 g, 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 - basal diet (Table 1) which prepared according to the following formula as mentioned by (AIN, 1993), The used vitamin mixture component (Table 2) was that recommended by (Campbell, 1963), while the salt mixture (Table 3) used was formulated according to (Hegsted , 1941).

After one week period, the rats were divided into two main groups, the first group (Group 1, 5 rats) still fed on basal diet and the other main group (30 rats) was feed with diet-induced obesity (DIO, product no.D1245, Research Diets, Inc. NJ, See (Table 4) for 4 weeks which classified into sex sub groups as follow: Group (+): fed on DIO as a positive control, Group (ppp), fed on DIO containing 5 % PPP, Group (CLP), fed on DIO containing 5 % CLP ,Group (ROSP), fed on DIO containing 5 % ROSP, Group (MPP), fed on DIO containing 5 % MPP and Group (Mixture): fed on DIO containing 5 % mixture, PPP + CLP+ ROSP + MPP by equal parts.

Table (1): basal diet according to (AIN, 1993).

Components	Gm. (%)
protein	10 %
Corn oil	10%
vitamin mixture	1%
mineral mixture	4%
methionine	0.3%
choline chloride	0.2%
cellulose	5%
corn starch	69.5%
total	100%

Table 4. Diet induced obesity (DIO) Formulae *
D12451 formula Research Diets, Inc. NJ. 2006

Components	Gm. (%)	Kcal (%)
Protein	24	20
Carbohydrate	41	35
Fat	24	45
Total		100
Casein, 80 Mesh	200	800
L-Cystine	3	12
Corn Strach	72.8	291
Maltodextrin 10	100	400
Sucrose	172.8	691
Cellulose ,BW200	50	0
Soybean Oil	25	255
Lard	177.5	1598
Mineral Mix S10026	10	0
DiCalcium phosphate	13	0
Calcium Carbonate	5.5	0
Potassium Citrate, 1 H2O	16.5	0
Vitamin Mix V10001	10	40
Choline Bitarrate	2	0
FD&C Red Dye #40	0.05	0
FD&C Blue Dye #1		
Total	858.15	4057

* Research Diets, Inc. NJ. 2006

Table (2): The composition of vitamin according to Campbell (1963)

compound	Amou
Vitamin A	200
Vitamin D	100
Vitamin E	10 I
Vitamin K	0.50
Vitamin B12	2.00
Thiamin	0.50
Pyridoxine	1.00
Niacin	4.00
Panthothenic acid	0.40
Choline chloride	200
Inositol	24 r
Folic acids	0.02
Para-amino benzoic acid	0.02
Biotin	0.02 mg

Table (3): Composition of minerals mixture according to Hegested et al., (1941).

Compounds	Amount (g)
$CaCO_3$	30.0
$KH2PO_4$	32.25
$CaHPO_4.H_2O$	7.50
$MgSO_4.7H_2O$	10.2
NaCl	16.66
$Fe(C_6H_2O_7).6H_2O$	2.75
KI	0.08
$MnSO_4.4H_2O$	0.50
$ZnCl_2$	0.025
$CuSO_4.5H_2O$	0.03
Total	100.0

2.2.4-Blood sampling

At the end of experiment period, blood samples were collected after 12 hours fasting 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 tubes and stored frozen at -20^{o}C until analysis.

2.2.5-Chemical Analysis:

Serum glucose was determined according to (Tinder, 1969). Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were measured in serum using the modified kinetic method of (Tietz, 1976) and serum alkaline phosphatase (ALP) activity was determined using modified kinetic method of (Vassault, *et al.*, 1999).

2.2.6- 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).

3- Results and Discussion

3.1- Results:

Table (5): The effect of plant by-product applied in bread on liver functions and plasma glucose in rat model of high fat diet induced obesity.

Parameters	Control (-)	Control (+)	Plant by-product (5%, w/w)				
			PPP	CLP	ROSP	MPP	Mixture
Serum AST	27.54±3.01 d	34.43±4.16 d	31.71±2.09 d	32.55±3 d	30.47±1.19 bc	30.56±2.9 d	29.31±4.87 d
Serum ALT	44.15±2.11 c	55.87±3.43 c	50.56±3.21 c	51.06±5.11 c	47.55±2.14 b	49.01±2.2 c	46.98±3.51 c
Serum ALP	89.15±5.23 b	110.14±8.67 b	100.54±3.49 b	102.65±6.5 b	96.9±6.98 a b	97.37±5.5 b	94.78±6.09 b
Plasma glucose	101.15±6.02 a	138.98±5.78 a	120.65±4.11 a	126.04±5.87 a	113.52±8.43 a	117.76±7.15 a	110.65±6.89 a

* Each value represents the mean of three replicates ±SD. Mean values with the different letters in the same column mean significantly different at level p≤0.05.

* 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

Control (+) induced a significant (p≤0.05) increase in AST (25.02%), ALT (26.55%), ALP (23.54%) and glucose (37.40%) when 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 AST, ALT and ALP activities and plasma glucose concentrations by the ratio of 7.90, 5.46, 11.50, 11.24 and 14.87; 9.50, 8.61, 14.89, 12.28 and 15.91; and 8.72, 6.80, 12.02, 11,59 and 13.95% ; 13.19, 9.31, 18.32, 15.27 and 20.38%, respectively when compared to controls (+).

The higher amelioration effect in serum glucose rising and the liver enzymes disorders induced by obesity in rats was recorded for the by-product mixtures treatment followed by ROSP, MPP, PPP and CLP, respectively.

3.2- Discussion:

Plant parts including ROSP, MPP, PPP are a rich source of different classes of phytochemicals such flavonols, phenolic acids, anthocyanins, alkaloids, carotenoids, phytosterols and organosulfur compounds (Beattic *et al.*, 2005 and Mohammed, 2012).

Flavonol glycosides reduced the elevated levels of the following serum enzymes, AST, ALT and ALP. Also, 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* El-Nashar, (2007).

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).

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 (Jung *et al.*, 2011).

Phenolic compounds present in 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 displays more efficient hypoglycemic action in alloxane-induced diabetic rats (Rodriguez *et al*., 1994).

These compounds are known for their properties in scavenging free radicals, inhibiting lipid peroxidation *in vitro* and improve glucose response and insulin resistance associated with type 2 diabetes (Jung *et al.*, 2011).

4. Conclusion:

The results indicated that, Supplementation of the rat diets with 5% w/w by PPP, CLP, ROSP, MPP and their mixture to ameliorate liver Functions and hyperglycemia in rat model of high fat diet induced obesity .These findings provide a basis for the use of plant by-products and also have important implications for the prevention and early treatment of obesity.

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