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Effect of Some Plant Parts Powder on Obesity Complications of Obese Rats

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Abstract:

The present study aims to investigate the effectiveness of some plant parts powder on obesity complications induced in experimental animals. Thirty male albino rats (150 ± 10g), were divided into two main groups, the first group (6 rats) still fed on basal diet and the other main group (contained 25 rats classified into five sub groups as follow: Group (2) fed on DIO (diet induced obesity) as a positive control; groups (from 3 to 6) fed on DIO containing 5% cauliflower leaves powder (CLP), mulberry leaves powder (MLP), pomegranates peel powder (PPP) and their mixture, respectively. The results stated that feeding on DIO leads to increase the BWG than the control group. At the end of the experiment (8 weeks), rats of the obese group recorded increasing rate 53.75 % of baseline. Replacement of carbohydrates diet with CLP, MLP, PPP and their mixture induced significant decreasing on BWG of the obese rats which recorded 31.26, 20.98, 7.49 and 5.68% of baseline, respectively. Biochemical analysis data indicated that obesity induced a significant increase in liver functions (28.99%, 30.82% and 29.61%U/L) as AST, ALT and ALP respectively. For serum lipid profile recorded (38 % mg/dlTG, 33.71 % mg/dl TC, 82.98 %mg/dl LDL-c and 38 % mg/dl VLDL,), serum glucose (29.91%) and MDA (Malonaldehyde, biomarker of serum lipid oxidation, 23.96 %) compared to normal control. Feeding on 5% of CLP, MLP, PPP 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 plant parts mixtures treatment followed by PPP, MLP and CLP.In conclusion, the present study has demonstrated the potency of plant parts including CLP, MLP, PPP and their mixture to ameliorate liver disorder and hyperglycemia in obese rats. Furthermore,CLP, MLP, PPP and their mixture lowered serum lipid peroxidation (malonaldehyde, MDA) levels and inflammatory stress in liver. These findings provide a basis for the use of CLP, MLP, PPP and their mixture for the prevention and early treatment of obesity and its complications.

Keywords: Cauliflower leaves, mulberry leaves, pomegranates peel, Biochemical analysis ,body weight gain, liver functions, serum lipid profile and serum glucose.

Introduction

Obesityis a state of excess adipose tissue mass. According to the Faculty of Public Health, obesity is "an excess of body fat frequently resulting in a significant impairment of health and longevity **(Nammi *et al.*, 2004).** Body fatness is most commonly assessed by body mass index (BMI) which is calculated by dividing an individual's weight measured in kilogram by their height in meter squared. Overweight is generally defined as a BMI greater than 25; individuals with a BMI greater than 30 are classified as obese. Obesity can be described as the "New World Syndrome". Its prevalence is on continuous rise in all age groups of many of the developed countries in the world. Statistical data reveals that the problem of obesity has increased from 12–20% in men and from 16–25% in women over the last ten years **(Callaway *et al.*, 2006)**. Recent studies suggest that nearly 15–20% of the middle aged European population are obese **(IOM, 2009)** and that in USA alone it is responsible for as many as 3,00,000 premature deaths each year **(Catalano and Ehrenberg , 2006).** Obese patients have been associated with increased risk of morbidity and mortality relative to those with ideal body weight **(Birdsall *et al.*,2009)**.

Obesity increases the risk of many physical and mental conditions. These comorbidities 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. The strength of the link between obesity and specific conditions varies. One of the strongest is the link with type 2 diabetes. Excess body fat underlies 64% of cases of diabetes in men and 77% of cases in women. Health consequences fall into two broad categories: Those attributable to the effects of increased fat mass (such as osteoarthritis, obstructive sleep apnea and social stigmatization) and the other due to the increased number of fat cells (diabetes, cancer, cardiovascular disease and non-alcoholic fatty liver disease). Increases in body fat alter the body's response to insulin, potentially leading to insulin resistance. Increased fat also creates a proinflammatory state and a prothrombotic state (**Bray, 2004).**

In more recent history, the use of plants as medicines has involved the isolation of bioactive compounds, beginning with the isolation of morphine from opium in the early 19th century. For example, many important bioactive compounds have been discovered from natural sources using bioactivity-directed fractionation and isolation. These bioactive compounds are mostly secondary plant metabolites, and many naturally occurring pure compounds have become medicine, dietary supplements and other useful commercial products.

Among plant materials/parts, processing of fruits and vegetables are resulting in high amounts of plants waste materials such as peels, seeds, stones, meals etc. industrialization of agriculturein the Arab worldrepresent a largeproportion ofwastewas estimated at18.14 milliontonnes per yearandrepresentremnants offruitandvegetables manufactureabout6.14% of this amount. Waste in the food industry is characterized by a high ratio of product-specific waste. This not only means that the generation of this waste is unavoidable, but also that the amount and kind of waste produced, which consists primarily of the organic residue of processed raw materials, can scarcely be altered if the quality of the finished product is to remain consistent. The utilization and disposal of product specific waste is difficult, due to its inadequate biological stability, its potentially pathogenic nature, its high water content, its potential for rapid autoxidation, as well as its high level of enzymatic activity. The diverse types of waste generated by various branches of the food industry can be quantified based upon each branches' respective level of production. Disposal of these materials usually represents a problem that is further aggravated by legal restrictions. Plant waste is prone to microbial spoilage; therefore, drying is necessary before further exploitation. The cost of drying, storage, and transport poses additional economical limitations to waste utilization. Therefore, agroindustrial waste often is utilized as feed or fertilizer. However, demand for feed or fertilizer varies and depends on agricultural production. Moreover, valuable nutrients contained in agroindustrial wastes are lost. Thus new aspects concerning 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. 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 **(Vasso and Constantina, 2007).**

Cauliflower *Brassica L. var. Botrytis* belongs to cruciferous family *Cruciferae*(*Brassicaceae*)which comprises also cabbage, broccoli, brussels sprouts, turnip, Swedish turnip.Cauliflower leaves which constitutes about 40-50% of cauliflower fruit considered as a waste by-product which obtained during processing (freezing and cooking) of cauliflower, huge amount of leaves is generated and its disposal is a major problem and causes environmental pollution.

Pomegranates (*Punicagranatum***,**L.) peels have been used extensively in the folk medicine of many cultures (**Longtin, 2003 and Yunfeng *et al.*, 2006).** Many studies found that pomegranate peel had the highest antioxidant activity among the peel, pulp and seed fractions of 28 kinds of fruits commonly consumed in China, as determined by FRAP (ferric reducing antioxidant power) assay **(Guo *et al.*, 2003**). Pomegranate peel may be a rich source of natural antioxidants and in the Middle East used as colorant for textiles because of their high tannin and phenolic contents. **Singh *et al.* (2002)** reported that methanol extract of pomegranate peel had much higher antioxidant capacity than that of seeds, as demonstrated by using the β-carotene–linoleate and DPPH model systems. Also, pomegranate peel extract could effectively protect (after oral administration) against CCl4 induced hepatotoxicity, in which ROS damage was intensively involved **(Murthy *et al.*, 2002).**

One of the less studied plants is the white mulberry (*Morus alba*, L.). It is a deciduous tree originating from Asia but currently cultivated in subtropical, tropical, and moderate environments (**Amarowicz *et al.*, 2000**). Different parts of the mulberry plant (fruit, bark, leaf and root) have been used over the centuries in traditional Chinese medicine as a common agent to treat a variety of conditions including diabetes, atherosclerosis, cancer as well as for boosting the immune system through potent antioxidant activity. The mulberry leaves are nutritious, palatable and nontoxic (**Srivastava *et al.*, 2003andButt *et al.*, 2008**). Several studies indicated that mulberry leaves contain many nutrients (e.g. proteins, dietary fiber and carbohydrates), minerals (e.g. iron, zinc, calcium, magnesium and phosphorous) and vitamins (e.g. ascorbic acid, β-carotene, B_1, D, and folic acid). Also, many bioactive compounds (e.g. flavonoids, phenolics acids, quercetin, isoquercetin and alkaloids) have been found in mulberry leaves (**Doi *et al.,* 2001**). Such bioactive compounds found in mulberry leaves possesses medical benefits, including diuretic, hypoglycemic, antibacterial, antiviral, hypotensive properties and neuroprotective functions (**Harauma *et al.,* 2007**).

Many studies reported that all of the previous plant parts are rich sources of bioactive compounds (vitamins: C, E and β-carotene, polyphenols, sulphur compounds, dietary fiber etc.). Varied bioactive components at different levels may be responsible for the offered health protection. 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 (**El-Sadany, 2001;Coskun *et al.,* 2005; Gorinstein *et al*., 2006; Taing *et al.,* 2012 and Matsunaga *et al.,* 2014).**

The present study aims to open new avenue for extending the using of three plant parts (pomegranate peel, mulberry leaves and cauliflower leaves) and their mixture in therapeutic nutrition through modulating obesity parameters using obese rat model. The effect of these plant parts and their mixture on liver and oxidant/antioxidant status, and serum glucose were also investigated in a trial to understand the basic concepts behind the pathogenesis of liver disease and diabetes in obesity.

Materials and Methods

Materials

Plant parts: Mullbery leaves, pomegranate fruits and cauliflowerleaves were obtained from Shebin El-Kom market, Menoufia Governorate, Egypt during the 2018 harvesting period. The collected samples was transported to the laboratory and used immediately for their powder preparation.

Wily mill: A wily mill (Tecator, Boulder, Co, USA) fitted with a 60 mesh screen sieve was used for grounding and sieving the all tested dried plant parts.

Basal diet: Basal diet constituents were obtained from El- Gomhoryia Company for Trading Drug, Chemical and Medical Instruments, Cairo, Egypt.

Rats: Animals used in this study, adult male albino rats (130±10 g per each) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Kits: For determination of liver functions, serum glucose, serum lipid profile, oxidative stress markers malondialdehyde (MDA), which were obtained from Biodiagnostic Co. Dokki, Giza, Egypt.

Chemicals, solvents and buffers: All chemicals, reagents and solvents were of analytical grade and purchased from Al-Gomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Methods

Preparation of plant parts powder

Pomegranate peel powder (PPP)

Unripe pomegranate peel were washed, sliced, pulp extraction 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 powder types.

Mulberry leaves powder (MLP) and cauliflower leaves powder (CLP)

Mulberry and 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˚C for 6 hrs followed by 40 ˚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.

Biological Experiments:

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 used vitamin mixture component was that recommended by **Campbell (1963)** while the salt mixture used was formulated according to **Hegsted (1941).**

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=30 rats), 130±10g per each, were housed individually in wire cages in a room maintained at 25 ± 2˚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 still fed on basal diet and the other main groups (25 rats) were feed with diet-induced obesity (DIO, product no.D1245, Research Diets, Inc. N.J.) for 8 weeks which classified into sex sub groups as follow: Group (2) fed on diet-induced obesity (DIO)as a positive control; group (3) fed on DIO containing 5 % CLP; group (4) fed on DIO containing 5 % MLP; group (5) fed on DIO containing 5 % PPP, and group (6) fed on DIO containing 5 % mixture, CLP+MLP+PPP by equal parts.

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.

Biological evaluation

During the period of the experiment, all rats were weighed once a week and the consumed diets were recorded everyday (daily feed intake). At the end of the experiment, biological evaluation of the experimental diets was carried out by determination of body weight gain (BWG) and feed efficiency ratio (FER) .

BWG (g) = Final weight (g) - Initial weight (g)

FER = Weight gain (g)/feed intake (g)

Hematological analysis

Liver functions

SGPT/ALT and **SGOT/AST** activities were measured in serum using the modified kinetic method of **Tietz *et al.* (1976)** by using kits supplied by Biocon. Company. Alkaline Phosphatase activity was determined using modified kinetic method of **Vassault *et al.* (1999)** by using kit supplied by Elitech Company .

Serum glucose

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

Serum 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

Malonaldialdehyde content (MDA)

Malonaldialdehyde content (MDA) content was measured as thiobarbituric acid reactive substances (TBARS) as described by **Buege and Aust (1978)**. Half milliliter of plasma were added to 1.0 ml of thiobarbituric acid reagent, consisting of 15% TCA, 0.375% thiobarbituric acid (TBA) and 0.01% butylatedhydroxytoluene in 0.25 N HCl. Twenty-five microliters of 0.1 M $FeSO_4.7H_2O$ was added and the mixture was heated for 20 min in boiling water. The samples were centrifuged at 1000 rpm for 10 min and the absorbance was read at 535 nm using Labo-med. Inc., spectrophotometer against a reagent blank. The absorbance of the samples was compared to a standard curve of known concentrations of malonaldehyde..

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

Effect of feeding of tested plant parts on FI (Feed intake), BWG (body weight gain) and FER (Feed efficiency ratio) ofobese rats

Effect of feeding tested plant parts on FI (Feed intake), BWG (body weight gain) and FER (Feed efficiency ratio) ofobese rats were shown in Table (1). From such data it could be noticed that the normal rats feeding with basal diet were recorded 22.05, 27.46 and 0.092 for FI, BWG and FER respectively. The obese rats (control positive) showed decreasing in FI and FER compared to normal rats by the ratio of -37.50 and -13.72%, respectively. On the other side there were increased in FI and FER by the all tested plant parts and their mixture when compared with the positive control group while were decreased in weight gain. The highest effects in manipulation of the FI, BWG and FER induced by obesity in rats were recorded for the plant parts mixture followed PPP, MLP and CLP, respectively. Such data are in accordance with that reported by **Sayed (2016)** who tested the breads blended with different agricultural processing by-products including potato, onion and cauliflower peels powder in obese rats. Also feeding of rats on diet induced obesity (DIO) leads to increase the body weight (BW) than the negative control group. At the end of the experiment (8 weeks), rats of the normal group recorded 188.02% of baseline for the BW while obese group was 231.07% of baseline. Replacement of wheat flour with potato , onion and eggplant peels powder and their mixture induced significant decreasing on BW of the obese rats which recorded 217.04, 203.03, 209.56 and 193.04% of baseline, respectively. The effect of different plant parts including PPP, YOSP, EPP and their mixture in the control of obesity is the main subjects of many studies (**Bedawy, 2008 and Bonet*et al.*, 2015**). 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 (**Rodriguez *et al*., 1994; Velioglu *et al*., 1998;Beattic *et al.*, 2005; Sayed, 2016 and Mashal, 2016)**. Such phytochemicals compounds and their metabolites have been shown to impact gene expression and cells (including adipocyte) function through different mechanisms including: 1) interacting with several transcription factors of the nuclear receptor superfamily, 2) interfering with the activity of other transcription factors, 3) modulating signaling pathways which are associated with inflammatory and oxidative stress responses; and 4) through extragenomic actions including scavenging of reactive oxygen and nitrogen species, **(Constance *et al.*, 2003; Bonet *et al.*, 2015; Mashal, 2016 and Sayed, 2016)** . All of these mechanisms could be contributed to their action control of adipocyte function, adiposity and obesity (**Bonet *et al.*, 2015**).

Table (1): Effect of feeding of tested plant parts on FI (Feed intake), BWG (body weight gain) and FER (Feed efficiency ratio) of obese rats

Groups	FI (g/d)		B W G (g/28d)		F E R	
	Mean	%of change	Mean	%of change	Mean	%of change
Group 1: Control Ve- (Normal)	22.05		27.46		0.092	
Group 2: Control Ve+ (Obese)	13.78	-37.50	42.22	53.75	0.072	-13.72
Group 3: Obese + CLP	16.33	-25.96	31.43	14.46	0.073	-1.33
Group 4:Obese + MLP	16.61	-24.67	33.22	20.97	0.077	-1.29
Group 5: Obese + PPP	16.74	-24.08	29.90	7.79	0.078	-5.11
Group 6: Obese + Mixture	17.44	-20.90	29.02	5.681	0.079	-1.07

Effect of feeding tested plant parts on serum liver enzymes activity ofobese rats

The effect of plant parts on liver function enzymes activity in plasma of obese rats were shown in Table (2). From such data, it could be noticed that obesity induced led to increase in AST (28.99%), ALT (30.82%) and ALP (29.61%) compared to normal controls. Adding of tested plant parts i.e. CLP, MLP, PPP and their mixture decreased the level of liver AST, ALT and ALP activities by the ratio of 21.53, 12.78, 11.86 and 9.12%; 25.85 for AST,25.85, 23.18, 18.08 and 17.95 for ALT and 17.93, 15.23, 12.01 and 7.03% for ALP respectively. The higher effects in manipulation of the liver enzymes disorders induced by obesity in rats were recorded for the plant parts mixture followed by PPP, MLP and CLP, respectively. Such data are in accordance with that reported by **Sayed (2016)** who tested the breads blended with different agricultural processing by-products including potato, onion and cauliflower peels powder in obese rats. Also, obesity induced a significant increased ($p \leq 0.05$) in AST (33.88%), ALT (25.62%) and ALP (26.76%) compared to normal controls. Replacement of wheat flour with potato , onion and eggplant peels powder and their mixture induced significant decreasing on liver AST, ALT and ALP activities by the ratio of 20.88, 13.73, 18.01 and 9.94; 13.47, 7.05, 11.01 and 4.80; and 17.03, 12.66, 16.31 and 10.22%, respectively.

Table (2): Effect of feeding tested plant parts on serum liver enzymes activity (U/L) of obese rats

Groups	Serum Aspartate aminotransferase activityAST		Serum alanine aminotransferase activityALT		Alkaline phosphatatse ALP	
	Mean	%of change	Mean	%of change	Mean	%of change
Group 1: Control Ve- (Normal)	75.89		27.77		161.67	
Group 2: Control Ve+ (Obese)	97.89	28.99	36.33	30.82	209.54	29.61
Group 3: Obese + CLP	92.23	21.53	34.95	25.85	190.66	17.93
Group 4:Obese + MLP	85.59	12.78	34.21	23.18	186.29	15.23
Group 5: Obese + PPP	84.89	11.86	32.79	18.08	181.09	12.01
Group 6: Obese + Mixture	82.81	9.12	32.76	17.95	173.05	7.03

Such as reviewed in several studies different plant parts including CLP, MLP, PPP and their mixture are a rich source of different classes of phytochemicals including flavonols, phenolic acids, anthocyanins, alkaloids, carotenoids, phytosterols and organosulfur compounds **(Harborne and Mabry, 1982;Velioglu *et al*., 1998; Singh *et al*., 2002;Beattic *et al*., 2005; Mohamed, 2012; Sayed, 2016 and Elhassaneen *et al*., 2016)**. The present study with others reported that the effect of different plant parts on decreasing the serum liver function enzymes activity could be attributed to their high level content of that phytochemicals. For example, active ingredients in sweet violet (*Viola odorata* L.) blossom powderprevented partially the rise of mean serum ALT, AST and ALP activities induced by CCl_4 injection **(Abd El-Fatah, 2013 and Elhassaneen *et al*., 2013)**. The potential mechanism of action of liver serum enzymes-lowering activity of the tested plant parts including CLP, MLP, PPP and their 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 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. 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* was reported by **El-Nashar (2007)**. Furthermore, 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)**.

Effect of feeding of tested plant parts on serum lipid profile of obese rats

The effect of plant parts on blood lipids profile of obese rats was shown in Tables (3 and 4). From such data it could be noticed that obesity induced led to increase in TG (38.00%), TC 33.71), LDL (82.98%) and VLDL (38.00)while it caused a decreasing in HDL (23.32%) compared to normal controls. Replacement of diet carbohydrates with 5% CLP, MLP, PPP and their mixture induced improvements on blood lipid profile through decreasing the TG,TC, LDL and VLDL by different ratios. 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 plant parts mixtures followed by PPP, MLP and CLP respectively. Data of the present study are in accordance with that observed by **Sayed (2016)** who tested the breads blended with different agricultural processing by-products including potato, onion and cauliflower peels powder in obese rats. Also, preclinical and clinical studies showed that mulberry leaves possessed various beneficial effects against cardiometabolic risks, including antihyperglycaemic, antihyperlipidaemic, antiobesity, antihypertensive, antioxidative, anti-inflammatory, anti-atherosclerotic and cardioprotective effects. Mulberry leaves could be a promising therapeutic option for modulating cardiometabolic risks. However, further investigations should be performed to substantiate the potential of mulberry leaves in practical uses. In the same context, modeling based on systematic reviews 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 HDL-c **(Avenell *et al.*, 2004; Bales &Buhr, 2008 ; Williamson *et al.*, 2009 and Ahmed, 2014).**

Table (3): Effect of feeding of tested plant parts on serum lipid profile (mg/dl) of obese rats

Groups	Total cholesterol (TC)		Triglycerides (TG)	
	Mean	**%of change**	**Mean**	**%of change**
Group 1: Control Ve- (Normal)	106.94		53.67	
Group 2: Control Ve+ (Obese)	142.99	33.71	74.07	38.00
Group 3: Obese + CLP	137.61	28.69	69.57	29.61
Group 4:Obese + MLP	130.70	22.22	65.21	21.51
Group 5: Obese + PPP	125.97	17.80	61.50	14.58
Group 6: Obese + Mixture	124.40	16.32	59.52	10.89

Table (4): Effect of feeding tested plant parts on lipoprotein cholesterol fractions (mg/dl) of obese rats

Groups	HDL-c		LDL-c		VLDL-c	
	Mean	**%of change**	**Mean**	**%of change**	**Mean**	**%of change**
Group 1: Control Ve- (Normal)	45.02		51.18		10.73	
Group 2: Control Ve+ (Obese)	34.52	-23.32	93.66	82.98	14.81	38.00
Group 3: Obese + CLP	36.33	-19.30	87.37	70.70	13.91	29.61
Group 4:Obese + MLP	37.90	-15.80	79.75	55.82	13.04	21.51
Group 5: Obese + PPP	38.93	-13.53	74.74	46.03	12.30	14.58
Group 6: Obese + Mixture	39.21	-12.91	73.29	43.18	11.90	10.89

In general, 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 LDL and TC in the blood are powerful risk factors for CHD, whereas high concentrations of HDL-c or a low LDL-c (or total) to HDL (**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 of several dietary components, such as found in our selected food processing by-products (EPP, PPP and their mixture) including, flavonols, phenolic acids, anthocyanins, alkaloids, carotenoids, phytosterols and organosulfur compounds etc have attracted much interest. Also, phenolic compounds found in such food processing by-products exert its beneficial effects on cardiovascular health by antioxidant and anti-inflammatory activities (**Kuhlmann *et al.*, 1998**). LDL oxidation and endothelial cell damage is believed to be involved in the early development of atherosclerosis **.** Researchers found that presence of phenolics significantly reduced LDL oxidation *in vitro* from various oxidases including 15-lipoxygenase, copper-ion, UV light, and linoleic acid hydroperoxide ***(*Kaneko *et al.,* 1994*)***.

Effect of feeding tested plant parts on serum glucose ofobese rats

Glucose concentration in serum of obese rats consumed plant parts was shown in Table (5). From such data it could be noticed that obesity caused an increaseing in serum glucose (99.56%) compared to normal controls. Supplementation of the rat dietswith 5% w/w by CLP, MLP and PPP and their mixture induceddecreasing in serum glucose concentrations by the ratio of 27.89, 25.34, 22.64 and 18.08%, respectively. The higher amelioration effect in serum glucose rising induced by obesity in rats was recorded for the plant parts mixtures treatment followed by PPP, MLP and CLP, respectively.

In similar studies, **Małgorzata, (2015)** found that the leaves of white mulberry (*Morus, alba* L.) functional food, due to the presence in its composition of valuable components, is beneficial to human health. Also, **Ewelina *et al.* (2016)** stated that mulberry leaves (*Morus alba*) have been used in folk medicine to mitigate symptoms of diabetes. The mulberry plant contains phenolic compounds that are able to decrease blood glucose concentration. Since various phenolics have antioxidant and metal binding properties, they can be used to alleviate oxidative stress and chelate trace elements involved in redox reactions. supplementation of the rat dietswith 5% w/w by potato, onion, eggplant and their mixture induced significant ($p \leq 0.05$) decreasing on serum glucose concentrations by the ratio of 12.36, 7.98, 10.62 and 4.96%, respectively. Furthermore, in patients with type II 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 potato, onion, cauliflower, mango peels powders was the subject of many studies **(Sayed, 2016)**. Moreover, onion skin powder (including the same bioactive compounds in the tested plant parts) 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. These findings provide a basis for the use of onion peel to improve insulin insensitivity in type 2 diabetes. Potato and pomegranate peel powders 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&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).** Finally, in the present study the mixture treatment gave maximum hypoglycemic yield when compared with the tested vegetables by-products separated. It could be mean that a combination of different plant parts may be more efficient for reducing the serum glucose level because the interactive effects occurred by different categories of bioactive compounds of the tested plant parts.

Table(5): Effect of feeding tested plant parts on serum glucose level (mg/dl) of obese rats

Groups	Glucose	
	Mean	**%of change**
Group 1: Control Ve- (Normal)	99.56	
Group 2: Control Ve+ (Obese)	129.34	29.91
Group 3: Obese + CLP	127.33	27.89
Group 4:Obese + MLP	124.79	25.34
Group 5: Obese + PPP	122.11	22.64
Group 6: Obese + Mixture	117.57	18.08

Effect of feeding tested plant parts on serum lipid peroxidation (malonaldehyde content, MDA) of obese rats

Data in Table (6) indicated the effect of tested plant parts on lipid peroxidation (malonaldehyde, MDA) concentration of obese rats. From such data it could be noticed that obesity increased the serum MDA (23.96%) compared to normal controls. Replacement of diet carbohydrates with 5% CLP, MLP, PPP and their mixture led to decrease MDA concentration by the ratio of 18.35, 17.23, 13.60 and 11.57%, respectively. In similar studies, the dried methanolic extract of tested plants was fed to albino rats of the Wistar strain, followed by carbon tetrachloride (CCl4), and the levels of various enzymes, such as catalase, peroxidase, and superoxide dismutase (SOD), and lipid peroxidation were studied. Treatment of rats with a single dose of CCl4 at 2.0 g/kg of body weight decreases the levels of catalase, SOD, and peroxidase by 81, 49, and 89% respectively, whereas the lipid peroxidation value increased nearly 3-fold. Pretreatment of the rats with a methanolic extract of pomegranate peel at 50 mg/kg (in terms of catechin equivalents) followed by CCl4 treatment causes preservation of catalase, peroxidase, and SOD to values comparable with control values, wheres lipid peroxidation was brought back by 54% as compared to control. Histopathological studies of the liver were also carried out to determine the hepatoprotection effect exhibited by the pomegranate peel extract against the toxic effects of CCl4 **(Ranasamy, 2014)**.

In the same context, clinical evidences for obesity-associated oxidative stress have been provided by measurement of either biomarkers or end-products of free radical-mediated oxidative processes. For instance, lipid peroxidation markers such as MDA, major products of the oxidation of polyunsaturated fatty acids, lipid hydroperoxides and conjugated dines are found to be increased in plasma from obese subjects in many clinical studies. Systemic metabolic alterations associated with obesity contribute to the increase in oxidative stress have been reported by many authors. For example, hyperglycemia as a hallmark of type II diabetes, a metabolic complication of obesity, induces oxidative stress through activation of the polyol and hexosamine pathways, production of advanced glycation end-products (AGE), and increasing of diacylglycerols (DAG) synthesis. Excess of circulating lipids induces ROS formation pathways, which contribute to the increase in lipid oxidation and protein carbonylation (**Sayed, 2016)**.

Table (6): Effect of feeding tested plant parts on serum lipid peroxidation (MDA, nmol/mg Hb) of obese rats

Groups	MDA	
	Mean	**%of change**
Group 1: Control Ve- (Normal)	233.29	
Group 2: Control Ve+ (Obese)	289.18	23.96
Group 3: Obese + CLP	276.11	18.35
Group 4:Obese + MLP	273.47	17.23
Group 5: Obese + PPP	265.02	13.60
Group 6: Obese + Mixture	260.28	11.57

Several years ago, interest in the possible significance of MDA on human health has been stimulated by reports that are mutagenic and carcinogenic compound. The positive effects of food processing by-products on oxidants formation/ concentration of obese rats could be attributed to several mechanisms induced by their bioactive components content **(Coskun *et al.*, 2005 and Sayed, 2016).** On the other side, the food processing by-products mixture treatment gave maximum reduction yield of plasma MDA when compared with the by-products individually. It could be mean that a combination of different food processing by-products may be more efficient for reducing plasma MDA level because the interactive effects occurred by different categories of bioactive compounds of the tested food processing by-products.

In conclusion, the present study has demonstrated the potency of plant parts including CLP, MLP, PPP and their mixture to ameliorate liver disorder and hyperglycemia in obese rats. Furthermore,CLP, MLP, PPP and their mixture lowered srum lipid peroxidation (malonaldehyde, MDA) levels i.e. suppressed oxidative and inflammatory stress in liver. These findings provide a basis for the use of CLP, MLP, PPP and their mixture for the prevention and early treatment of obesity and its complications.

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تأثير بعض مساحيق الأجزاء النباتية على مضاعفات مرض السمنة فى الفئران

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الملخص العربى :
تهدف الدراسة الى إستكشاف تأثير بعض الأجزاء النباتية على مضاعفات مرض السمنة المستحث فى الفئران. تم تقسيم ثلاثون فأر (130±10جم) إلى مجموعتين رئيسيتين، المجموعة الأولى تم تغذيتها على الغذاء الأساسي (مجموعة ضابطة سالبة) والمجموعة الرئيسية الأخرى (25 فار) تم تغذيتها علي نظام غذائي يسبب السمنة (DIO) لمدة 8 أسابيع، تم تقسيمها فيما بعد إلى خمسة مجموعات فرعية على النحو التالي: المجموعة (2) تم تغذيتها على غذاء (DIO) كمجموعة ضابطة موجبة، اما المجموعات (3 - 6) تم تغذيتها علي غذاء DIO يحتوي على 5٪ من مسحوق ورق القرنبيط (CLP)، مسحوق ورق التوت (MLP)، مسحوق قشور التفاح (PPP) ومسحوق مخلوطهم، على التوالي. وتم تغذية الفئران على نظام غذائي يسبب السمنة لمدة ثمانية أسابيع ادي إلى زيادة وزن الجسم المكتسب بمقدار 53.75% (مقارنة بلحظة بداية التجربة). بينما استبدال كربوهديرات الوجبة الغذائية للفئران بنسبة 5٪ من CLP, MLP, PPP ومخلوطهم ادي الي حدوث انخفاض كبير في اوزان الفئران التي تعاني السمنة وذلك بنسب 31.26, 20.98, 7.49 and 5.68% على التوالي. أشارت النتائج أن السمنة ادت الي زيادة معنوية في كل من وظائف الكبد (AST بنسبة 28.99%، ALT بنسبة 30.82%، ALP بنسبة 29.61%,، صورة دهون الدم (الجليسريدات الثلاثية بنسبة 38%والكوليستيرول نسبة 33.71%والليبوبروتينات منخفضة الكثافة بنسبة 82.98% ، والليبوبروتينات منخفضة الكثافة جدا بنسبة 38%) والجلوكوز في مصل الدم بنسبة 29.91% والمالونالدهيد بنسبة 23.96% مقارنة بالمجموعة الضابطة السالبة. كما وجد ان التغذية على 5٪ من CLP, MLP, PPP ومسحوق مخلوطهم ادي الي تحسن ملحوظ في كل التقديرات السابقة وبنسب مختلفة. وقد سجلت افضل التأثيرات لمخلوط الأجزاء النباتية ثم مسحوق فشور الرمان، مسحوق ورق التوت، مسحوق ورق القرنبيط علي التوالي. وخلصت الدراسة ان الأجزاء النباتية التى تم دراستها لها انعكاسات مهمة للوقاية والعلاج المبكر من مضاعفات السمنة مثل ارتفاع سكر الدم، تحسين صورة دهون الدم، وتقليل مستوى أكسدة دهون الدم.

الكلمات المفتاحية:قشور الرمان، ورق التوت، ورق القرنبيط ،المنتجات الثانوية ،الزيادة فى وزن الجسم ، وظائف الكبد ، سكر الدم، المالونادهيد،التحاليل الحيويه،التحاليل الكيميائيه.