Effect of cabbage and radish leaves on obesity biological changes induced in rats

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Abstract

The present study aims to investigate the effect of cabbage and radish leaves on obesity biological changes induced in rats. Thirty male albino rats (140±9.56 per each), were divided into two main groups, the first group (Group 1, 5 rats) still fed on basal diet and the other main group (40 rats) classified into seven sub groups as follow: group (2), fed on DIO as a positive control; groups (3-8), fed on DIO containing cabbage leaves powder (CLP) and radish leaves powder (RLP) by the ratio of 2.5, 5.0 and 7.5%, respectively. 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 180.11 % of baseline for the BW while obese group was 228.43% of baseline. Replacement of wheat flour with CLP and RLP induced significant ($p \leq 0.05$) decreasing on BW of the obese rats which recorded 203.03, 190.78 and 188.79% (CLP) and 217.04, 214.00 and 209.56% of baseline for the ratio of 2.5, 5.0 and 7.5%, respectively. Biochemical analysis data indicated that obesity induced a significant increase in serum glucose, serum lipid profile (TG, TC, LDL and VLDL) and serum acetylcholine esterase compared to normal controls. Feeding on 2.5, 5.0 and 7.5%, of CLP and RLP exhibited a significant improvement ($p \leq 0.05$) in all of these parameters by different rates. The amelioration effects were higher in CLP than RLP. In conclu-

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sion, these results provide a basis for the use of the tested plant parts (CLP and RLP) in an important therapeutic feeding application which is the prevention and early treatment of obesity.

Keywords: cabbage, radish, leaves powder, body weight, serum glucose, lipid profile, acetylcholine esterase.

Introduction

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have adverse effects on health (Haslam and James, 2005). According to the Faculty of Public Health in US, obesity is "an excess of body fat frequently resulting in a significant impairment of health and longevity (Nammi, *et al.*, 2004). Also, obesity is due to a loss of the balance between energy intake and expenditure over long periods of time, and the brain plays a critical role in controlling and inhibiting the pre-potent responses to foods (Morton *et al.*, 2014). Obesity is most commonly assessed by body mass index (BMI) which is equal to weight/height^2 (in kg/m^2). Individuals with a BMI greater than 30 are classified as obese. Most academic authorities are using the term *overweight* rather than obese to describe individuals with BMIs between 25 and 30. Such rates of BMI should be viewed as medically significant and worthy of therapeutic intervention, especially in the presence of risk factors that are influenced by adiposity, such as hypertension and glucose intolerance.

Based on data of substantial morbidity, a BMI of 30 is most commonly used as a threshold for obesity in both men and women. Large-scale epidemiologic studies suggest that all-cause, metabolic, cancer, and cardiovascular morbidity begin to rise (albeit at a slow rate) when BMIs are $\geq$25, suggesting that the cut-off for obesity should be lowered (Elhassaneen and Salem, 2015). Increasing in body weight is correlated with different diseases, including cardiovascular diseases, diabetes mellitus type 2, obstructive sleep apnea, certain types of cancer, osteoarthritis and asthma. As a result, obesity has been found to reduce life expectancy (Haslam and James, 2005). 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). Also, obesity is a risk factor for several malignancies including colon, pancreatic, thyroid, hepatic, and uterine cancer and for cardiovascular diseases, and diabetes mellitus (Alzahrani *et al*., 2014; and Mokdad, 2003). Furthermore, recent studies reported that obesity can induces several complications including oxidative stress, hyperglycemia, immunological parameters deficiency and neurological disorders (Almutairiu, 2020).

Survey studies have declared that consumption of different plant parts such as vegetables imparts health benefits, e.g. reduced risk of coronary heart disease, stroke, diabetics, obesity and certain types of cancer (Jin and Kyung, 2001and Banihani, 2017). Also, Yeh and Yen, (2005) shown that consumption of vegetables was associated with the prevention of chronic diseases such as cancer and cardiovascular disease. Furthermore, the intake of 400-600 g/d of fruits and vegetables was associated with reduced incidence of many common forms of cancer and also associated with a reduced risk of heart disease and many chronic diseases of aging (Heber, 2004). They also mentioned that consumers are advised to daily ingest one serving of each of the seven colour groups, putting this recommendation within the United States National Cancer Institute and American Institute for Cancer Research guidelines of five to nine servings per day. Apart from dietary fiber, these health benefits are mainly attributed to many organic micronutrients including phytochemicals, vitamins and others. These foods contain phytochemicals that have anti-cancer and anti-inflammatory properties, which could confer many health benefits. Other mechanism could be related to the induction of cellular phase II detoxifying enzymes was associated with cancer preventive potential (Yeh and Yen, 2005). So, the study of Heimendinger and Chapelsky (1996) reported that a minimum of five servings a day of vegetables and fruits, especially of green and yellow vegetables and citrus fruits, is recommended. Although consumers are increasingly aware of diet related health problems, a large group of the population lacks a generous intake of fruits and vegetables. Thus, dietary supplements and food fortification may be an alternative route to the consumption of minor plant components that may have health benefits. We will limit our study here to two *cruciferaceae* family vegetables, cabbage and radish most commonly produced by a large quantities in Egypt.

Cabbage (*Brassica oleracea* L) is a green leafy vegetable that belongs to the *brassica* family, a group of vegetables including cabbage, collards and brussels sprouts that have widespread attention due to their health promoting, containing phytonutrients. Polyphenols in cabbage are flavonoids (mainly flavonols), the major polyphenolic constituents of cabbage, flavonols such as quercetin and kaempferol, indole-3-carbinol and isothiocyanate (Rice-Evans *et al.*, 1996). Red color is derived mainly from a class of flavonoids called anthocyanins. Anthocyanin concentrations ranged from 1111 to 1780 mg Cy3G/100 g DM (Neda *et al.*, 2014). There is also an increasing interest in anthocyanins because of their potential health-promoting properties and, above all, for their protection against free radicals. Also, Ragaa *et al.*, (2019) reported that red radish contains several natural compounds that have the antioxidant potent such as phenolic compounds and pigments that might lower oxidative stress and hepatic protective. Protective effect of cabbage phytochemicals was confirmed against cancer, cardiovascular disease, diabetes, obesity, aging conditions etc (Ho and Chang , 2006; Buko *et al.*, 2018 and Vugic *et al.*, 2020).

Radish (*Raphanus sativus*) belong to *cruciferaceae* family, and is grown as well as consumed all over the world, widely in India for its culinary and medicinal purposes and considered part of the human diet (Eskin and Tamir, 2005). The root crops were a common food in Egypt long before the pyramids were built; it is mainly sowed from September to March, harvested within 30-50 days of sowing and is pulled out from the soil when it reaches edible size (Abdel Magied *et al.*, 2016). Red radish is rich in protein, sugars, carbohydrates, dietary fibers and some fat and fluoride (Khattak, 2011 and Abdel Magied *et al.*, 2016). In addition, it contains minerals including Ca, Fe, Mg, Zn, P, and K about 25, 0.34, 10, 0.28, 20 and 233 mg/100g, respectively and various water-soluble vitamins including B1, B2, B6, and C about 0.012, 0.039, 0.071 and 14.8 mg/100g, respectively (USDA, 2016). On the other side, red radish roots are content phenolic acids such as ferulic, sinapic, catechin, caffeic, gallic, syringic, rutin and gentisic acid. Meanwhile, coumaric acid was the most abundant phenolic acid that recorded 0.520 g/100g followed by vanillic acid (0.410 g/100g.) Also, radish contains flavonoids such as kaempherol glycosides, peroxidases and antioxidants (Suh *et al.*, 2006 and Hashimoto *et al., 2*006). Furthermore, radish root contains a significant quantity of anthocyanins, which are water-soluble natural pigments belong to the flavonoids class of compounds and that have the strongest antioxidant power out of 150 flavonoids (Choi *et al.*, 2009). Additionally, it contains glucosinolates, glucoraphanin, glucoraphanin,4-hydroxyglucobrassicin, glucoerucin, glucoraphasatin, glucobrassicin, 4-methioxyglucobrassicin, neoglucobrassicin, isothiocyanates, sulforaphene, sulforaphane, and indole-3-carbinol which conceder unique bioactive compounds and have been recently recognized to have potential health benefits to humans (Baenas *et al.*, 2016). Radish recommended for the treatment and prevention of diseases such as cardiovascular disease and cancer, jaundice, gallstone, liver diseases, rectal prolapse, indigestion, and other gastric pains (Banihani, 2017; Jin and Kyung, 2001).

In an atrial to open new horizons for the use of these plants in important therapeutic nutrition fields, the present study was conducted with the aim of exploring the effect of *Brassicaceae* family vegetables, namely cabbage and radish on obesity biological changes induced in rats.

Materials and Methods

Materials

Vegetables, red cabbage and radish were obtained from a local market of Benha City, Benha Governorate, Egypt. The collected samples was transported to the laboratory and used immediately for leaves powder preparation process.

Wheat flour and baking materials (salt and dried yeast) were purchased from Benha City market, Benha Governorate, Egypt.

Casein was obtained from Morgan Chemical Co., Cairo, Egypt. All minerals and vitamins in food grade used for rats feeding, chemicals, reagent and buffers, all in analytical grade, were purchased (except for the aforementioned next to some of the items in their places) from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. Specific analytical kits used for biological assays were purchased from the aforementioned companies corresponding to each.

Methods

Cabbage (CLP) and radish (RLP) leaves powder preparation

Cabbage and radish 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 powder that passed through an 70 mesh sieve was retained for use.

Preparation of Balady bread

The Balady bread samples were prepared according to the used in Elhassaneen *et al.,* (2016-a) with some modifications. In brief, formulation of the bread is applied as follow: wheat flour, 1000 g; salt, 20g; and dried yeast, 2 g; and water 500 g. Yeast was mixed with water (30 ^{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 cabbage leaves powder (CLP) and radish leaves powder (RLP), were conducted based on 2.5, 5.0 and 7.5% of the weight of the wheat flour. The dough was later proofed for 3 hours in a proofer (Bakbar E81, New Zealand), then cut into loafs 80 g prior to baking at 175 ^{0} C for 8 min.

Sensory evaluation of bread

Sensory evaluation was carried out with 10 panelists comprising of postgraduate students from BenhaUniversity, Benha, Egypt. Each panelist was served with 4 randomly arranged bread samples on a rectangular plastic tray. The loafs were individually sealed in a pouch and coded with a three-digit number prior to testing. The 4 samples consisted of 6 types of composite flour loafs and a control (100% wheat flour). Water was provided for rinsing between the samples. Panelists were required to evaluate the color, taste and overall acceptance of the bread using the 9-point hedonic scale with 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, and 9=like extremely.

Biological Experiments

Animals

Animals used in this study, adult male albino rats (140±9.56 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 used vitamin mixture component (Table

1) was that recommended by (Campbell, 1963) while the salt mixture (Table 2) 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=40 rats), were housed individually in wire cages in a room maintained at 26 ± 4 ^{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, 5 rats) still fed on basal diet and the other main group (35 rats) was feed with diet-induced obesity (DIO, product no.D1245, Research Diets, Inc. NJ, See Table 3) 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 2.5% CLP, Group (4), fed on DIO containing 5.0% CLP, Group (5), fed on DIO containing 7.5% CLP, Group (6), fed on DIO containing 2.5% RLP, Group (7), fed on DIO containing 5.0% RLP, and Group (8), fed on DIO containing 2.5% RLP. Body weight gain (BW, gram and as percent of the 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

Serum glucose

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

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

Acetylcholinestrase (AChE) determination

Serum AChE activity (Rappaport units/mL) was assayed according to the colorimetric method mentioned by Gowenlock, (1988).

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

Sensory evaluation of bread incorporated with selected plant parts

Data in Table (1) show the results of sensory evaluation in terms of colour, taste and overall acceptance of bread incorporated with selected plant parts. The CLP and RLP breads were significantly different ($p \leq 0.05$) in colour, taste and overall acceptance. The ROSP bread was shown to be the least liked by the panelists. The initial acceptance of baked products is much influenced by colour, which can also be an indicator of baking completion. As selected plant parts flour imparts relatively a dark brown colour to the breads, this might have given the panelists an impression of 'overbaked' product, thus affecting their likings. For colour, among the selected plant parts, the colour of CLP breads was rated the highest (7.20) by the panelists. The desirable colour of breads is mainly due to the Maillard browning during baking. However, in CLP breads, the colour could be partially contributed by the anthocyanin's in CLP flour which imparts a redwish colour to the breads. Similar observations were reported in a study by Sayed Ahmed (2016) and El-Harbi, 2018) who observed that an increased flour and thus muffin visual lightness (with more yellowness and brownness rather than dark and yellow green) yield a higher aroma, texture and colour acceptability scores. On the other side, significant ($p \leq 0.05$) difference was observed in terms of taste between the control, and the tested plant parts (CLP and RLP) breads. This could probably be due to the nature of CLP and RLP which impart an additional flavour to the breads. Data of the sensory evaluation with the chemical, physical and rheological properties of the breads incorporated with the selected plant parts recommended the using of such product as an important functional food and could be potentially introduced in many therapeutic nutrition applications.

Table 1. Sensory evaluation of the control and composite flour breads

Parameters	Control bread (CB)	CB + 7.5 CLP	CB + 7.5 RLP
Colour (Scores)	8.41 ± 0.30 [a]	7.20 ± 0.18 [b]	7.01 ± 0.13 [b]
Taste (Scores)	8.03 ± 0.21 [a]	7.20 ± 0.23 [b]	6.85 ± 0.19 [bc]
Overall acceptance (Scores)	8.71 ± 0.19 [a]	7.31 ± 0.24 [b]	7.04 ± 0.12 [b]

* Each value represents the mean of twelve replicates ±SD. Mean values with the different letters in the same row mean significantly different at level $p \leq 0.05$.

The effect of cabbage and radish leaves powder applied in bread on body weight gain (BWG, g) of obese rats

The effect of cabbage and radish leaves powder applied in bread on body weight gain (Percent of change from the baseline) of obese rats was shown in Table (2) and Figure (1).Such data indicated that feeding of rats on diet induced obesity (DIO) leads to increase the body weight (BW) than the control group. At the end of the experiment (8 weeks), rats of the normal group recorded 182.05% of baseline for the BW while obese group was 231.07% of baseline. Supplementation of diets with cabbage (CLP) and radish (RLP) leaves powder by the ratio of 2.5, 5.0 and 7.5% exhibited significant ($p \leq 0.05$) decreasing on BW of the obese rats by the rate of 203.03, 190.78 and 188.79%; and 217.04 ، 214.00 and 209.56% of baseline, respectively. The higher effect on weigh decreasing was recorded for CLP than RLP. Such data are in accordance with that observed by many authors (Kim *et al.,* 2014; Bertoia *et al.,*2016; Sayed ahmed, 2016; and Ghozy and Tag Al Deen, 2020).

The positive effects of such selected plant parts regarding the control of the obesity could be attributed to their high level content of different categories of phytochemicals including phenolics, anthocyanins, carotenoids, phytosterols, indoles, alkaloids and organosulfur compounds (Bahorun *et al.,* 2004; Qi *et al.,* 2005; Hashimoto *et al.,* 2006; Suh *et al.,* 2006; Choi *et al.,* 2009; Rosario *et al.,* 2015). The possible mode of action of weight-lowering activity (anti-obesity) of the tested bread supplemented with selected plant parts (CLP and RLP) could be explained by one or more of the following mechanisms. Such phytochemicals groups have been shown to impact gene expression and cell (including adipocyte) function through one or several of the following mode if action, interacting with several transcription factors of the nuclear receptor superfamily, interfering with the activity of other transcription factors, modulating signaling pathways which are associated with inflammatory and

Table (2): The effect of cabbage and radish leaves powder applied in bread on body weight gain (Percent of change from the baseline) of obese rats*

Groups	Feeding period (weeks)								
	0	1	2	3	4	5	6	7	8
Control (-) Std diet	100.00	106.20	114.76	120.67	136.78	156.56	168.67	174.80	182.05 ^{e}
Control (+) Obese	100.00	118.03	133.65	140.41	173.73	200.93	204.66	216.71	231.07^{a}
CLP (2.5%)	100.00	109.24	122.40	126.75	147.80	174.96	178.20	186.71	203.03 ^{bc}
CLP (5.0%)	100.00	109.91	118.87	125.85	142.67	163.02	174.86	178.78	190.78 ^{d}
CLP (7.5%)	100.00	107.49	116.25	123.08	139.54	159.44	171.01	177.92	188.79 ^{d}
RLP (2.5%)	100.00	115.79	127.52	133.38	155.54	190.87	198.05	203.40	217.04 ^{b}
RLP (5.0%)	100.00	114.17	125.74	131.52	153.36	185.00	194.00	200.55	214.00 ^{b}
RLP (7.5%)	100.00	112.89	122.84	129.18	149.36	190.21	194.71	196.98	209.56 ^{bc}

* RLP, radish leaves powder, CLP, cabbage leaves powder. Values in the same row with different superscript letters are significantly different at $p \leq 0.05$.

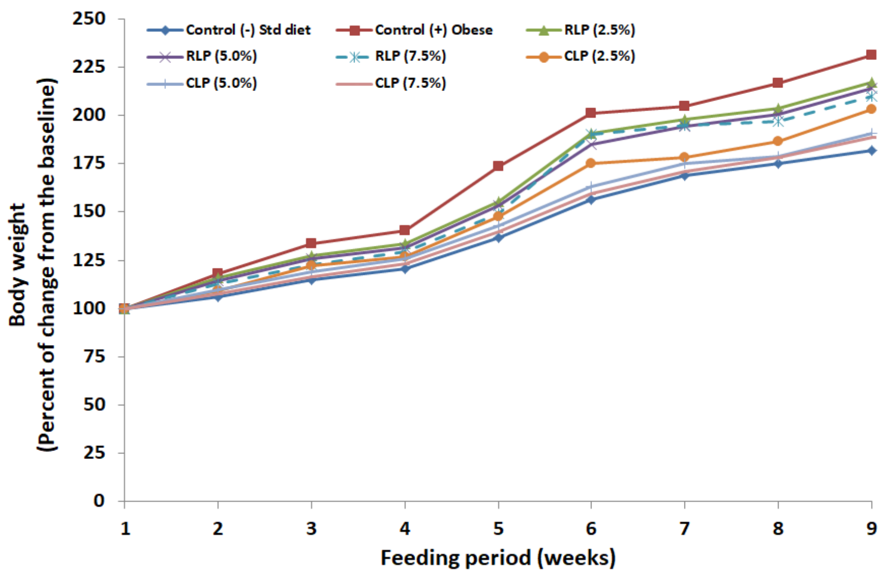

Figure (1): The effect of cabbage and radish leaves powder applied in bread on body weight gain (Percent of change from the baseline) of obese rats*

* CLP, cabbage leaves powder; RLP, radish leaves powder. Values in the same row with different superscript letters are significantly different at $p \leq 0.05$.

oxidative stress responses, and extra-genomic actions such scavenging of reactive species (Constance *et al.,* 2003; Bray, 2004; Bonet *et al.,* 2015, Elmaadawy *et al.,* 2016 and Sayed Ahmed, 2016). Also, red radish sprout extract (RRSE) abolished adipocyte differentiation and lipid and triglyceride accumulation without cytotoxicity in 3T3-L1 adipocytes, modulated the expression of the proteins related to adipogenic transcription factors: peroxisome proliferator-activated receptor (PPAR)γ, sterol regulatory element-binding protein 1 (SREBP-1), and CCAT/enhancer binding protein (C/EBP)α. RRSE also suppressed expression of the proteins responsible for lipid synthesis, transport, and storage: adiponectin, fatty acid synthesis (FAS), perilipin, and fatty acid bind protein-4 (FABP4). That is meaning RRSE treatment has the potential to inhibit obesity by controlling the expression of adipogenic transcription factors and adipogenic proteins (Kim and Kim., 2014). The anthocyanins and a pro-anthocyanidins which dominant pigments in CLP and RLP help to maintain body weight and prevent obesity and its associated consequences in healthy adults (Bertoia *et al.,* (2016). additionally, anthocyanins (found in red cabbage and radish) exhibited anti-obesity activity *in vitro*. At the concentration of 250–1000 g/mL, anthocyanins significantly suppressed the preadipocyte proliferation (up to 75.51%) and decreased the total lipid accumulation (up to 69.62% (Chaiittianan *et al.,* 2017). Furthermore, Anna *et al.,* (2017) reported that relations between enzymes involved in carbohydrate and lipid digestion (α-amylase, α-glucosidase, and lipase) and phenolic compounds and anthocyanin's levels in red cabbage. Finally, Vugic *et al.,* 2020) recommended that the regular intake of anthocyanins reduced obesity-associated inflammation in overweight/obese subjects. Subsequently, all of these mechanisms participate to their action control of adipocyte functions, adiposity and obesity.

Serum glucose levels of obese rats feeding of cabbage leaves powder (CLP) and radish leaves powder (RLP) applied in bread

The effect of selected plant powder (CLP and RLP) applied in bread on plasma glucose in serum of obese rats were shown in Tables (3 and 4) and Figures (2 and 3). From such data it could be noticed that obesity induced a significant increased ($p \leq 0.05$) in serum glucose (32.69%) compared to normal controls. Replacement of wheat flour with cabbage leaves powder (CLP) by the ratio of 2.5, 5.0 and 7.5% induced significant ($p \leq 0.05$) decreasing on serum level glucose (as the percentage of change from the negative control/standard diet group) by the ratio of 24.23, 15.65 and 3.05%, respectively. The same behavior was observed for the radish leaves powder (RLP) but at a slightly lower rate. Supplementation of the diet with 2.5, 5.0 and 7.5% of RLP induce decreasing on serum glucose level (as the percentage of change from the negative control/standard diet group) by the rates of 27.69, 22.19 and 8.52%, respectively. Therefore, it is noticed that treatment of obesity complications (hyperglycemia) has risen by increasing the level of the selected plant parts (CLP and RLP) added to the diet. Such data are in according with that reported by Gaafar *et al.,* (2014) who investigated the comparative potential and ameliorative effect of both white and red cabbages (*Brassica oleracea*) in the management of STZ induces diabetes in rats. Diabetes was induced in male Westar rats using streptozotocin; STZ, 60 mg/kg body weight. Oral supplementation of white and red cabbage extracts to diabetic rats resulted in significant change in serum glucose.

Data of the present study with the other reported that serum glucose may be elevated significantly ($p \leq 0.05$) in additionally induced diabetic disease such as obesity in both human and experimental animals (Elhassaneen and Salem, 2015; Sayed Ahmed, 2016 and Almutairiu, 2020). Our present data with the others found that the selected plant parts, CLP and RLP, are a rich source of different categories of phytochemicals including phenolics, anthocyanins, alkaloids, carotenoids, phytosterols and organosulfur compounds (Rice-Evans *et al.*, 1996, Bahorun *et al.*, 2004; 2004; and Neda *et al.*, 2014). Many studies reported that the effect of CLP and RLP on decreasing the serum glucose level could be attributed to their high level content of these compounds. For example, Gaafar *et al.*, (2014) who investigated the comparative potential and ameliorative effect of both white and red cabbages (*Brassica oleracea*) in the management of STZ induces diabetes in rats. Oral supplementation of white and red cabbage extracts to diabetic rats resulted in

Table 3. Serum glucose concentration (mg/dl) of obese rats feeding of cabbage leaves powder applied in bread*

Value	Control (-) Std diet	Control (+) Obese	Cabbage leaves powder (CLP, w/w)		
			2.5	5.0	7.5
Range	86.62-103.84	117.12-140.41	111.04-131.15	98.46-118.05	92.93-111.41
Mean	95.62^{b}	126.89^{a}	118.80^{a}	110.59^{ab}	98.54^{b}
SD	5.56	8.11	11.78	7.87	6.45
% of change	0.00	32.69	24.23	15.65	3.05

* Values in the same row with different superscript letters are significantly different at $p \leq 0.05$.

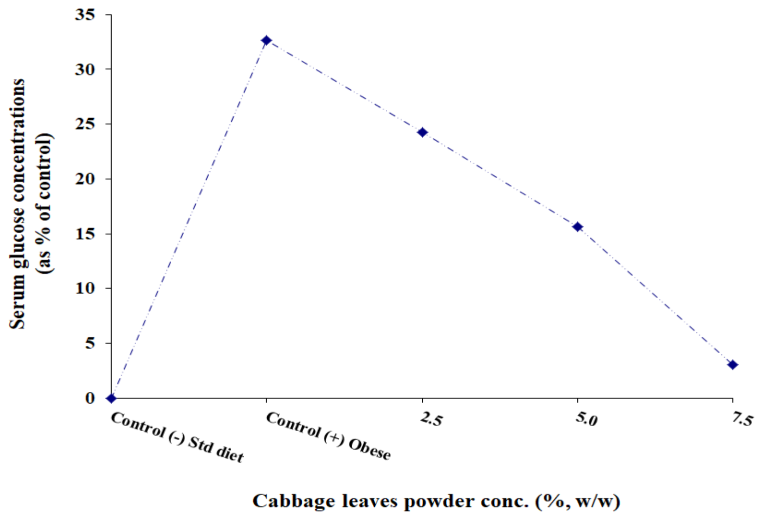

Figure 2. Serum glucose concentration (as % of control) of obese rats feeding of cabbage leaves powder (CLP) applied in bread*

significant change in serum glucose. Also, Saleem, (2017) reviewed that radish has been identified as having antidiabetic effects, making it favorable for those with diabetic conditions. This may be due to its ability to enhance the antioxidant defense mechanism and reduce the accumulation of free radicals, affect hormonal-induced glucose hemostasis, promote glucose uptake and energy metabolism, and reduce glucose absorption in the intestine. Furthermore, Buko *et al.,* (2018) evaluated the protective effect of red cabbage extract (RCE) in rats with streptozotocin-induced diabetes, assessing a probable role of this extract in the prevention of erythrocyte impairments associated with a high risk of vascular complications in diabetes. The RCE treatment lowered blood glucose, and glycated and fetal hemoglobin concentrations and improved glucose tolerance as well as considerably raised serum insulin, proinsulin and C-peptide levels in streptozotocin-treated rats. Simultaneously, RCE improved pancreatic islet morphology, increasing the amount of pancreatic β-cells in diabetic animals. On the other side, many plant parts content phytochemicals found in CLP and RLP displays potent hypoglycemic action in alloxane-induced diabetic rats (Sayed Ahmed, 2016 and El-Harbi, 2018). 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, since these bioactive compounds are present in a greater number and quantity in cabbage powder, this clearly explains why CLP is effective in lowering blood sugar to a greater degree than RLP.

Table 4. Serum glucose concentration (mg/dl) of obese rats feeding of radish leaves powder applied in bread*

Value	Control (-) Std diet	Control (+) Obese	Radish leaves powder (RLP, w/w)		
			2.5	5.0	7.5

Range	86.62-103.84	117.12-140.41	111.85-137.10	103.64-127.25	92.67-112.10
Mean	95.62[b]	126.89[a]	122.11[a]	116.84[ab]	103.77[b]
SD	5.56	8.11	5.09	7.78	6.44
% of change	0.00	32.69	27.69	22.19	8.52

* Values in the same row with different superscript letters are significantly different at p≤ 0.05.

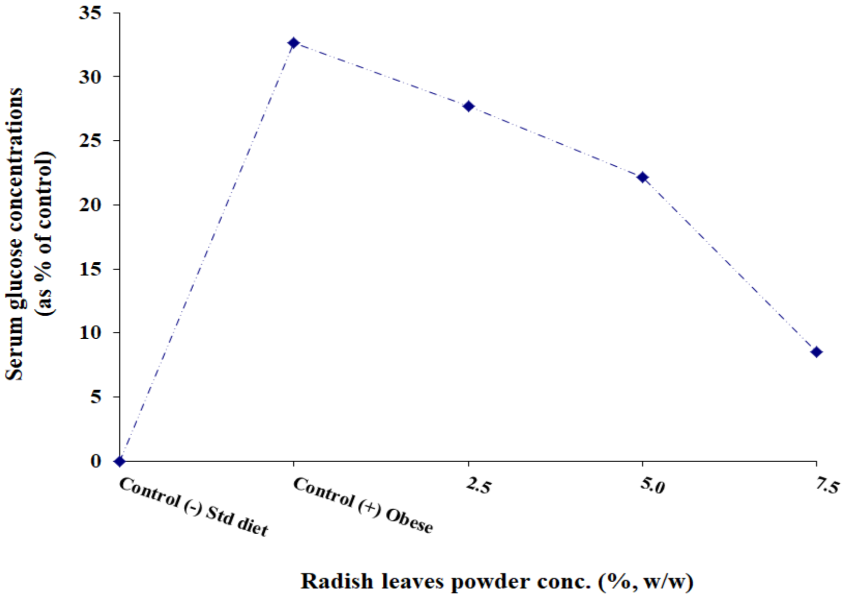

Figure 3. Serum glucose concentration (as % of control) of obese rats feeding of radish leaves powder (RLP) applied in bread*

Blood lipids profile of obese rats feeding of cabbage (CLP) and radish (RLP) leaves powder applied in bread

The effect of selected plant powder (CLP and RLP) applied in bread on blood lipids profile of obese rats was shown in Tables (5 and 6) and Figures (4 and 5). From such data it could be noticed that obesity induced a significant increased (p≤0.05) in triglycerides (TG, 50.21), total cholesterol (TC, 33.39), low density lipoprotein

(LDL, 68.58) and very low density lipoprotein (VHDL, 50.21) compared to normal controls. Replacement of wheat flour with cabbage leaves powder (CLP) by the ratio of 2.5, 5.0 and 7.5% induced significant ($p \leq 0.05$) decreasing on serum TG, TC, LDL and VHDL levels (as the percentage of change from the negative control/standard diet group) by the ratio of 29.29, 15.73 and 7.69%; 19.45, 13.46 and 4.04%; 39.04, 26.93 and 4.49; and 29.69, 15.73 and 7.69%, respectively. The same behavior was observed for the radish leaves powder (RLP) but at a slightly lower rate. Supplementation of the diet with 2.5, 5.0 and 7.5% of RLP induce decreasing on serum TG, TC, LDL and VHDL level(as the percentage of change from the negative control/standard diet group) by the rates of 39.19, 21.50 and 10.45%; 4.21, 6.90 and 6.01%; 51.18, 32.28 and 8.98%; and 39.19, 21.50 and10.45%, respectively. The opposite direction was recorded for the High density lipoprotein (HDL). Obesity induced a significant ($p \leq 0.05$) decrease in HDL (-16.08%) compared to normal controls. Replacement of wheat flour with cabbage leaves powder (CLP) by the ratio of 2.5, 5.0 and 7.5% induced significant ($p \leq 0.05$) increasing on serum HDL levels (as the percentage of change from the negative control/standard diet group) by the ratio of -8.32, -4.46 and 2.55% (for CLP) and -10.40, -5.38 and -0.39% (for RLP), respectively. Such data are in accordance with Banihani, (2017); and Jin and Kyung, (2001) who mention that radish recommended for the treatment and prevention of diseases such as cardiovascular disease. It can decrease the plasma TC, TG and phospholipids in normal rats. Furthermore, Gaafar *et al.,* (2014) found that oral supplementation of white and red cabbage extracts to diabetic rats resulted in insignificant change in serum total lipids level, TC, TG and HDL. LDL showed significant decrease as compared to normal control rats.

Therefore, it is noticed that treatment of obesity complications (impaired serum lipid protein levels) has risen by increasing the level of the plant parts (CLP and RLP) added to the diet. In this mode, 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 TC and LDL in the blood are powerful risk factors for CHD, whereas high concentrations of HDL cholesterol or a low LDL cholesterol or (reviewed in Bedawy, 2008 and ElMaadawy *et al.*, 2016). 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 (phyto-bioactive compounds) such as found in our selected plant parts (CLP and RLP) including phenolics, anthocyanins, alkaloids, carotenoids, phytosterols and organo-sulfur compounds have attracted much interest . For example, Heber, (2004) reported that diets rich in plant foods (vegetables, fruits and phytoestrogens) were also associated with a reduced risk of heart disease and many chronic diseases of ageing. These foods contain phytochemicals that have anti-cancer and anti-inflammatory properties, which could confer many health benefits. Also, Jayanta *et al.,* (2012) found that anthocyanin-rich red cabbage extract (ARCE) treatment of rats fed the atherogenic (ATH) diet significantly prevented elevation in serum and tissue lipids, circulating levels of cardiac and hepatic damage markers, and resulted in excretion of lipids through faces.

The possible mode of action of serum lipid particles-lowering levels of the tested bread supplemented with selected plant parts (CLP and RLP) could be explained by one or more of the following mechanisms. Phenolics found in selected plant parts exert its beneficial effects on cardiovascular health by antioxidant and anti-inflammatory activities (Anonymous, 1998 and Kuhlmann *et al.,* 1998; Elbasouny *et al.*, 2019; Almutairiu, 2020 and Sayed, 2020). LDL oxidation and endothelial cell damage is believed to be involved in the early development of atherosclerosis (Kaneko *et al.,* 1994; and Bedawy, 2008). Researchers found that presence of phenolics such quercetin found in the selected plant parts 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). Besides the direct

Table 5. Blood lipids profile concentration of obese rats feeding of cabbage leaves powder applied in bread*

Value	Control (-) Std diet	Control (+) Obese	Cabbage leaves powder (CLP, w/w) 2.5	5.0	7.5
Triglycerides (TG, mg/dL)					
Range	44.44-53.28	68.03-81.55	60.49-71.89	50.71-60.79	49.83-59.73
Mean	49.07[cd]	73.70[a]	63.64[b]	56.79[c]	52.84[cd]
SD	3.42	5.21	6.23	5.03	4.33
% of change	0.00	50.21	29.29	15.73	7.69
Total cholesterol (TC, mg/dL)					
Range	89.90-107.78	122.20-146.51	112.59-130-22	100.56-120.56	97.38-116.75
Mean	99.25[c]	132.39[a]	118.55[b]	112.61[b]	103.26[c]
SD	4.78	6.87	5.44	6.22	6.11
% of change	0.00	33.39	19.45	13.46	4.04
High density lipoprotein (HDL, mg/dL)					
Range	35.43-44.47	30.30-36.32	30.28-43.94	33.27-39.89	37.83-45.35
Mean	39.11[a]	32.82[b]	35.86[ab]	37.37[a]	40.11[a]
SD	3.12	4.01	4.22	4.52	4.39
% of change	0.00	-16.08	-8.32	-4.46	2.55
Low density lipoprotein (LDL, mg/dL)					
Range	45.58-54.64	78.30-93.87	63.21-81.09	56.87-68.18	49.59-59.44
Mean	50.32[c]	84.83[a]	69.97[b]	63.88[b]	52.58[c]
SD	5.24	6.22	8.45	9.45	5.21
% of change	0.00	68.58	39.04	26.93	4.49
Very low density lipoprotein (VHDL, mg/dL)					
Range	8.89-10.65	13.61-16.31	10.10-15.17	10.11-12.12	9.97-11.94
Mean	9.81	14.74	12.73	11.36	10.57
SD	0.76	2.04	1.78	2.06	3.02
% of change	0.00	50.21	29.69	15.73	7.69

* Values in the same row with different superscript letters are significantly different at p≤ 0.05.

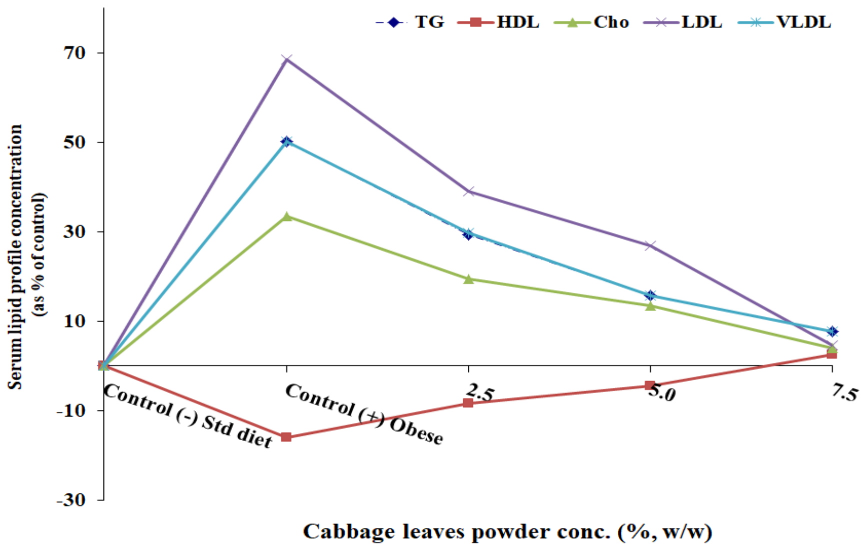

Figure 4. Blood lipids profile concentration (as % of control) of obese rats feeding of cabbage leaves powder (CLP) applied in bread*

Table 6. Blood lipids profile concentration of obese rats feeding of radish leaves powder applied in bread*

Value	Control (-) Std diet	Control (+) Obese	Radish leaves powder (RLP, w/w) 2.5	5.0	7.5
Triglycerides (TG, mg/dL)					
Range	44.44-53.28	68.03-81.55	62.56-74.99	52.88-63.39	48.39-58..01
Mean	49.07^{c}	73.70^{a}	68.29^{a}	59.62^{b}	54.19^{b}
SD	3.42	5.21	4.11	3.89	5.10
% of change	0.00	50.21	39.19	21.50	10.45
Total cholesterol (TC, mg/dL)					
Range	89.90-107.78	122.20-146.51	111.30-137.04	102.45-122.83	94.45-113.03
Mean	99.25^{d}	132.39^{a}	124.78^{b}	115.50^{c}	104.64^{d}
SD	4.78	6.87	4.21	6.90	6.01
% of change	0.00	33.39	25.73	16.37	5.43
High density lipoprotein (HDL, mg/dL)					
Range	35.43-44.47	30.30-36.32	32.10-39.48	32.82-40.35	34.79-43.71
Mean	39.11^{a}	32.82^{ab}	35.05^{ab}	37.01^{ab}	38.96^{a}
SD	3.12	4.01	5.22	3.78	4.65
% of change	0.00	-16.08	-10.40	-5.38	-0.39
Low density lipoprotein (LDL, mg/dL)					
Range	45.58-54.64	78.30-93.87	69.69-83.55	59.04-70.78	48.97-58.71
Mean	50.32^{d}	84.83^{a}	76.08^{b}	66.57^{c}	54.84^{d}
SD	5.24	6.22	5.99	6.10	4.58
% of change	0.00	68.58	51.18	32.28	8.98
Very low density lipoprotein (VHDL, mg/dL)					
Range	8.89-10.65	13.61-16.31	12.51-15.00	10.57-12.68	9.68-11.60
Mean	9.81^{ab}	14.74^{a}	13.66^{a}	11.92^{a}	10.84^{ab}
SD	0.76	2.04	1.99	2.05	2.11
% of change	0.00	50.21	39.19	21.50	10.45

* Values in the same row with different superscript letters are significantly different at $p \leq 0.05$.

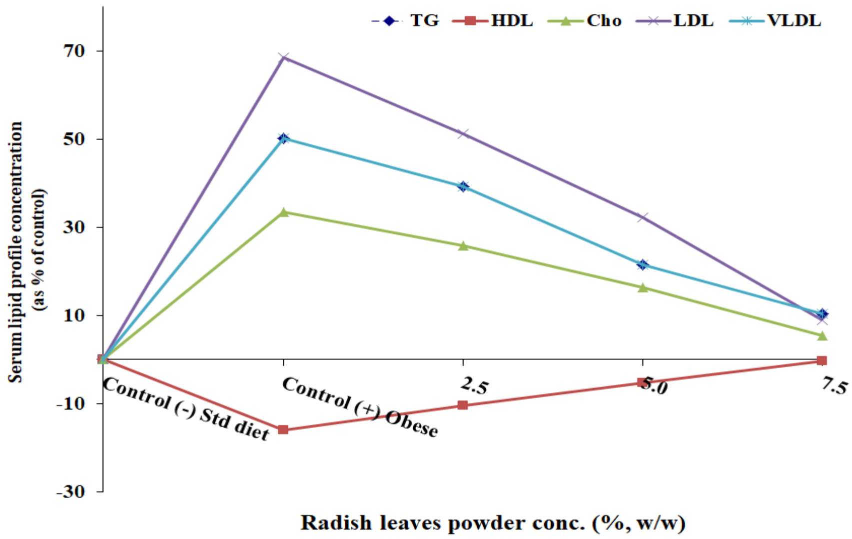

Figure 5. Blood lipids profile concentration (as % of control) of obese rats feeding of radish leaves powder (RLP) applied in bread.

Serum acetylcholine esterase (AChE) activity of obese rats feeding of cabbage leaves powder (CLP) and radish leaves powder (RLP) applied in bread

The effect of selected plant powder (CLP and RLP) applied in bread on serum AChE activity of obese rats was shown in Tables (7 and 8) and Figures (6 and7). From such data it could be noticed that obesity induced a significant increase ($p \leq 0.05$) in serum AChE (22.20%) compared to normal controls. Replacement of wheat flour with cabbage leaves powder (CLP) by the ratio of 2.5, 5.0 and 7.5% induced significant ($p \leq 0.05$) increasing on serum AChE activity (as the percentage of change from the negative control/standard diet group) by the ratio of 16.85, 12.67 and 4.89%, respectively.The same behavior was observed for the radish leaves powder (RLP) but at a slightly lower rate. Supplementation of the diet with 2.5, 5.0 and 7.5% of RLP induce decreasing on serum AChE activity (as the percentage of change from the negative control/standard diet group) by the rates of 19.35, 15.40 and 8.01%, respectively. Therefore, it is noticed that treatment of obesity complications (neurological disorders) has risen by increasing the level of the selected plant parts (CLP and RLP) added to the diet. Such data are in according with that reported by Meharam *et al.,* (2021) who investigated the effect of selected food processing byproducts (onion skin, banana and apricot stone powders) in serum AChE activity of obese rats. They indicated that obesity induced a significant ($p \leq 0.05$) increasing in serum AChE which partially ameliorated by treatment with these food processing byproducts.

AChE is an acetylcholine hydrolyzing enzyme that is responsible for the termination of cholinergic response. An increased AChE activity in brain encouraged memory deficits as well as oxidative stress (OS) (Melo *et al.,* 2003). In the present study, obesity induced increase in AchE activity which confirmed in several previous studies (Paes et al., 2006 and Zahra *et al.*, 2017). They found that long-term of pomegranate extracts feeding reduce the AChE activity. The increasing of AChE activity could be attributed to calcium ions (Ca^{2+}) influx-mediated OS caused by Amyloid beta peptides. Our data with the other explain the mechanism of anticholinesterase activity of selected plant parts which may be due to antioxidant activities of their bioactive compounds such phenolics, anthocyanin's, alkaloids and carotenoids. Such compounds protect of neural cell from OS insults by different mechanisms. One of these mechanisms is preventing the influx of Ca2+ despite high levels of ROS, increasing intracellular GSH and directly lowering levels of ROS (Ishige *et al.,* 2001). These findings highlight the neuroprotective effects of selected plant parts and one of mechanisms is the inhibition of AChE and the stimulation of antioxidant activities. Additionally, CLP is effective in lowering serum AChE activity to a greater degree than RLP which could be attributed to the present of bioactive compounds in greater number and quantity in cabbage powder.

Table 7. Serum acetylcholine esterase (ACE, Rappapor U/mL) of obese of obese rats feeding of cabbage leaves powder applied in bread*

Value	Control (-) Std diet	Control (+) Obese	Cabbage leaves powder (CLP, w/w)		
			2.5	5.0	7.5
Range	69.89-70.89	69.67-78.64	66.67-77.45	65.64-71.67	63.67-70.56
Mean	62.01^{b}	75.78^{a}	72.46^{a}	69.87^{ab}	65.04^{b}
SD	5.56	6.78	4.56	5.12	3.96
% of change	0.00	22.20	16.85	12.67	4.89

* Values in the same row with different superscript letters are significantly different at $p \leq 0.05$.

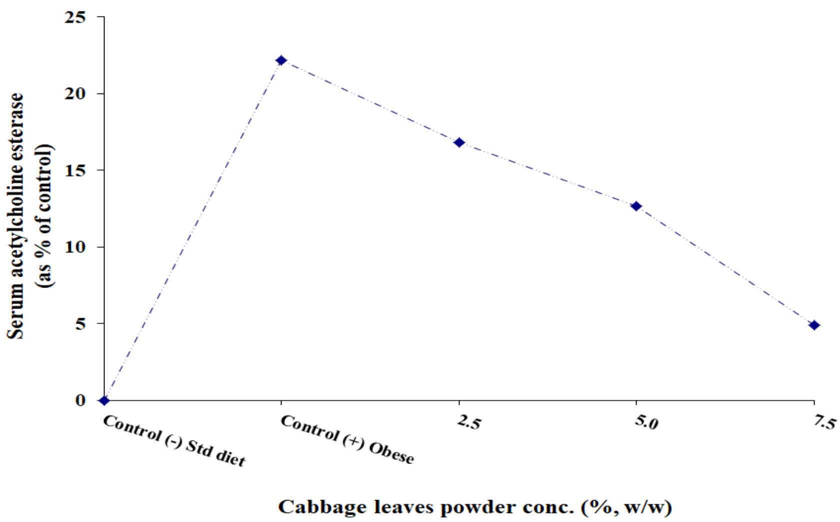

Figure 6. Serum acetylcholine esterase (ACE, as % of control) of obese of obese rats feeding of cabbage leaves powder (CLP) applied in bread.

Table 8. Serum acetylcholine esterase (ACE, Rappapor U/mL) of obese of obese rats feeding of radish leaves powder applied in bread*

Value	Control (-)	Control (+)	Radish leaves powder (RLP, w/w)

	Std diet	Obese	2.5	5.0	7.5
Range	69.89-70.89	69.67-78.64	65.87-78.56	67.45-73.65	61.45-69.67
Mean	62.01 ^{b}	75.78^{a}	74.01^{a}	71.56 ^{ab}	66.98 ^{b}
SD	5.56	6.78	4.45	3.56	2.78
% of change	0.00	22.20	19.35	15.40	8.01

* Values in the same row with different superscript letters are significantly different at p≤ 0.05.

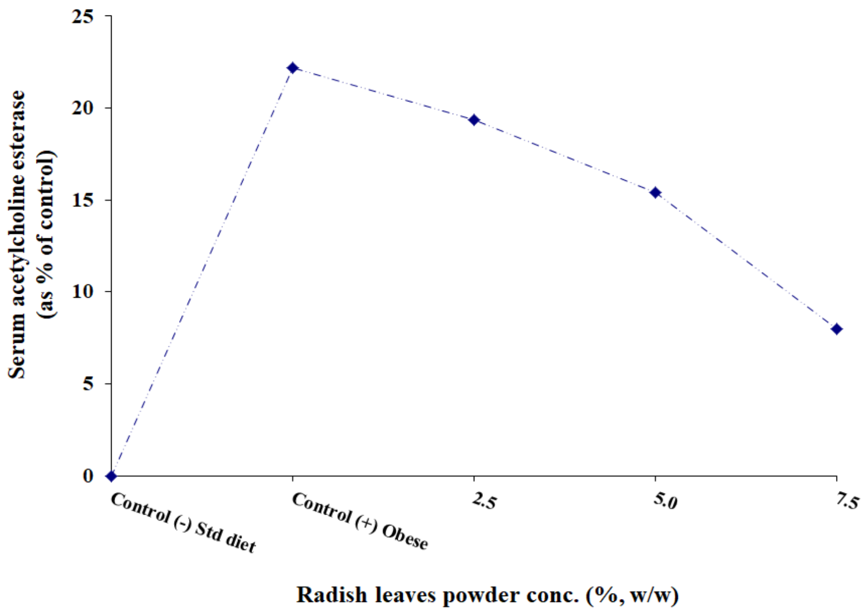

Figure 7. Serum acetylcholine esterase (ACE, as % of control) of obese of obese rats feeding of radish leaves powder (RLP) applied in bread.

In conclusion, the present study has demonstrated the potency of the selected plant parts including CLP and RLP to ameliorate hyperglycemia, serum lipid profile and acetylcholine esterase activity (as a marker of nerodisorders) in obese rats. These findings provide a basis for the use of CLP and RLP and also have important implications for the prevention and early treatment of obesity. Furthermore, more research must be done on the future in the area of plant parts with the high content of different bioactive compounds classes and extended their applications in human diets and food technology applications.

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تأثير أوراق الكرنب والفجل على التغيرات البيولوجية لمرض السمنة المستحث في الفئران

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تهدف الدراسة الحالية إلى معرفة تأثير أوراق الكرنب والفجل على التغيرات البيولوجية للسمنة التي تحدث في الفئران. لذلك، تم تقسيم أربعون من ذكورفئران الألبينو (١٤٠ ± ٩,٥٦ جرام لكل فأر) إلى مجموعتين رئيسيتين، المجموعة الأولى (مجموعة ١، ٥ فئران) تم تغذيتها على الغذاء الأساسي (مجموعة ضابطة سالبة) والمجموعة الرئيسية الأخرى (٣٥ فار) تم تغذيتها علي نظام غذائي يسبب السمنة (DIO) لمدة ٨ أسابيع، تم تقسيمها فيما بعد إلى سبعة مجموعات فرعية على النحو التالي: المجموعة (٢) تم تغذيتها على غذاء (DIO) كمجموعة ضابطة موجبة، اما المجموعات (٣ - ٨) تم تغذيتها علي غذاء DIO يحتوي على مسحوق أوراق الكرنب (CLP) ومسحوق أوراق الفجل (RLP)بنسبة ٢,٥ و ٥,٠ و ٧,٥٪ على التوالي. تغذية الفئران على نظام غذائي يسبب السمنة لمدة ثمانية أسابيع ادي إلى زيادة وزن الجسم عند مقارنتها بالمجموعة الضابطة وقد سجلت المجموعة الضابطة السالبة زيادة فى الوزن مقدارها ١٨٠,١١٪ ، فى حين سجلت المجموعة الضابطة الموجبة زيادة فى الوزن مقدارها ٢٢٨,٤٣٪ مقارنة بلحظة بداية التجربة. أدى استبدال دقيق القمح باستخدام CLP و RLP إلى انخفاض معنوي ($p \leq 0.05$)على وزن الجسم للجرذان التي تعاني من السمنة والتي سجلت ٢٠٣,٠٣ و ١٩٠,٧٨ و ١٨٨,٧٩٪ (CLP) و ٢١٧,٠٤ و ٢١٤,٠٠ و ٢٠٩,٥٦٪ من خط الأساس عند الاستبدال بنسبة ٢,٥ و ٥,٠ و ٧,٥٪ على التوالي. أشارت بيانات التحليل البيوكيميائي إلى أن السمنة تسببت في زيادة معنوية في نسبة جلوكوز الدم وصورة الدهون فى الدم(الجليسريدات الثلاثية ، الكوليستيرول الكلى، الليبوبروتينات منخفضة الكثافة، الليبوبروتينات منخفضة الكثافة جدا) ودرجة نشاط انزيم الأسيتايل كولين استيريز مقارنة بالضوابط العادية. أظهرت التغذية على CLP, RLP بنسب ٢,٥ و ٥,٠ و ٧,٥٪ الى حدوث تحسنًا معنويا ($p \leq 0.05$) في كل هذه المقاييس السابقة بمعدلات مختلفة. كما أظهر مسحوق CLP تأثيرات أعلى مما سجل فى مسحوق RLP . وفي النهاية ، توفر نتائج هذه الدراسة أساسًا علميا لاستخدام أجزاء النبات المختبرة (CLP و RLP) في احدى تطبيقات التغذية العلاجية الهامة وهو الوقاية والعلاج المبكر للسمنة .

الكلمات المفتاحية: الكرنب ، الفجل ، مسحوق الأوراق ، وزن الجسم ، جلوكوز الدم، صورة دهون الدم ، أستيل كولين استريز.