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 على وظائف الكبد ومستوي السكر في الدم للفئران التي تم تغذيتها على نظام غذائي عالي الدهون مسبب للسمنة.

By
Sara Ahmed Mohamed Sayed Ahmed
Assistant lecture in Home Economics Department, Faculty of Specific Education, Port Said University.

Prof. Dr.
Yousif Abd El-Aziz Elhassaneen
Professor and Head Department of Nutrition and Food Science Ex-Dean Faculty of Home Economics, Minoufiya University.

Prof. Dr.
Safaa Ali El-Wasseef
Assistant Professor of Nutrition and Food Science in Home Economics Depart., Faculty of Specific Education, Port Said University.

Dr.
Naglaa Mohamed Fathy
Lecturer in Home Economics Department, Faculty of Specific Education, Port Said University.
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.
The effect of Plant by-products on liver functions and plasma glucose in rats fed a high fat diet induced obesity

Sara Ahmed Mohamed Sayed Ahmed

The study highlights the effect of using plant by-products (potato peel and Opuntia, onion peel, mango peel) in nutritional therapy through evaluating the addition of meals containing these by-products to a high-fat diet in reducing obesity and examining their effect on liver function and plasma glucose in rats affected with obesity. The study suggests a natural method to reduce obesity and its related diseases such as diabetes and liver disease. The study shows that the rats affected with obesity had a significant increase in blood glucose and liver enzyme activity compared to the control group. Adding meals containing these by-products to the high-fat diet resulted in a significant decrease in blood glucose and liver enzyme activity. The addition of a mixture of the by-products resulted in an improvement in these parameters compared to the rats affected with obesity. In summary, the results of the study indicate that using plant by-products can help reduce obesity and its related diseases.
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 modifications, 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

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

Sara Ahmed Mohamed Sayed Ahmed

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 Negil, Kom Hamada, Behira Government, Egypt. Mangoes peel (Mangifera indica L. cv Copania): fruits were obtained from a local farm, Ismailia 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 °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 °C for 12 and 40 °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 °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.

2.2.2 - Preparation of Balady bread:

Formulation of the bread is applied as follow: wheat flour, 1000 g; salt,
The effect of Plant by-products on liver functions and plasma glucose in rats fed a high fat diet induced obesity

Sara Ahmed Mohamed Sayed Ahmed

20g; and dried yeast, 2 g; and water 500 g. Yeast was mixed with water (25°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 loaves 120 g prior to baking at 170°C for 10 min.

2.2.3 - Experimental design

All biological experiments performed must comply 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°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 fed 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).

<table>
<thead>
<tr>
<th>Components</th>
<th>Gm. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>protein</td>
<td>10 %</td>
</tr>
<tr>
<td>Corn oil</td>
<td>10%</td>
</tr>
<tr>
<td>vitamin mixture</td>
<td>1%</td>
</tr>
<tr>
<td>mineral mixture</td>
<td>4%</td>
</tr>
<tr>
<td>methionine</td>
<td>0.3%</td>
</tr>
<tr>
<td>choline chloride</td>
<td>0.2%</td>
</tr>
<tr>
<td>cellulose</td>
<td>5%</td>
</tr>
<tr>
<td>corn starch</td>
<td>69.5%</td>
</tr>
<tr>
<td>total</td>
<td>100%</td>
</tr>
</tbody>
</table>
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 aspirated, transferred into clean tubes and stored frozen at -20°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).
The effect of Plant by-products on liver functions and plasma glucose in rats fed a high fat diet induced obesity

Sara Ahmed Mohamed Sayed Ahmed

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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (-)</th>
<th>Control (+)</th>
<th>Plant by-product (5%, w/w)</th>
<th>PPP</th>
<th>CLP</th>
<th>ROSP</th>
<th>MPP</th>
<th>Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum AST</td>
<td>27.54±3.01</td>
<td>34.45±4.10</td>
<td>31.71±2.09</td>
<td>32.55±3.07</td>
<td>30.47±1.19</td>
<td>30.56±2.92</td>
<td>29.31±4.87</td>
<td></td>
</tr>
<tr>
<td>Serum ALT</td>
<td>44.15±3.11</td>
<td>55.87±4.31</td>
<td>50.56±3.21</td>
<td>51.06±5.11</td>
<td>47.55±2.14</td>
<td>49.01±2.22</td>
<td>46.98±3.51</td>
<td></td>
</tr>
<tr>
<td>Serum ALP</td>
<td>89.15±5.23</td>
<td>110.14±8.67</td>
<td>100.54±3.49</td>
<td>102.65±6.50</td>
<td>96.9±6.98</td>
<td>97.37±5.50</td>
<td>94.78±6.09</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>101.15±6.02</td>
<td>138.98±5.78</td>
<td>120.64±4.11</td>
<td>126.04±5.87</td>
<td>113.52±8.43</td>
<td>117.76±7.15</td>
<td>110.65±6.89</td>
<td></td>
</tr>
</tbody>
</table>

* 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.

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 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 byproduct 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
The effect of Plant by-products on liver functions and plasma glucose in rats fed a high fat diet induced obesity
Sara Ahmed Mohamed Sayed Ahmed

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.
5. References:


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

Sara Ahmed Mohamed Sayed Ahmed


