Evaluation of Carrot Pomace (Daucus carota L.) as Hypocholesterolemic and Hypolipidemic Agent on Albino Rats

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Abstract

The current study examined the attenuating influence of dietary carrot pomace powder (CaPP) on hypercholesterolemia and various oxidative stress-associated with biochemical parameters in hypercholesterolemic rats. Thirty two male albino rats weighing 110±10 g were divided into four groups, the first group received the basal diet only and served as (negative control), the second group received the hypercholesterolemic diet and served as positive control, the other groups received hypercholesterolemic diet supplemented with 10%, 20% CaPP for six weeks. The obtained results revealed that groups supplemented with 10% and 20% CaPP significantly decrease total lipid, total cholesterol, triglycerides, low density lipoprotein cholesterol, liver enzymes: alanine aminotransferase, aspartate aminotransferase compared to positive and negative groups. Organs weight, body weight gain significantly decreased compared with positive control. Moreover dietary carrot pomace powder can used to reduce the body weight and reducing hypercholesterolemic complications. In addition, dietary carrot pomace powder serves to improve the blood picture and to reduce the blood glucose level in hypercholesterolemic rats and could use in obese people for body loss. Data of kidney function (Urea) record an increase in CaPP 20% level (26.9±2.96) but this increase was non significant with the negative control group (26.6±3.1).

Keywords: Daucus carota, hypercholesterolemia, kidney function, obese people

Introduction

Although cholesterol is important and necessary for mammals, but high levels of cholesterol in the blood can damage arteries and are potentially linked to diseases such as those associated with the cardiovascular system and heart disease (Ingelsson et al., 2007).

Fiber is often classified according to its solubility in water into soluble and insoluble dietary fibers (Schnee- man, 2007; Van Way and Ireton-Jones, 2004). Increased dietary fiber intakes are associated with significantly lower prevalence rates of cardiovascular disease (Graham et al., 2007). Soluble fiber, when included within a low saturated fat and cholesterol diet, lowers low-density lipoprotein cholesterol concentration about 5-10% in hypercholesterolemic and diabetic patients (Anderson et al., 2009). A high intake of fiber, particularly of the soluble type, above the level recommended (25-35 g/day) by the American Dietetic Association (ADA), improves glycemic control, decreases hyperinsulinemia, and lowers plasma lipid concentrations (Chandalia et al., 2000).

Waste by-products of vegetable food processing represent a major disposal problem in industry (Schieber et al., 2001). Its transformation into value added products, as fibers may contribute to diminish the problem and to recover valuable biomass and nutrients (Gerschenson et al., 2009).

Carrot (Daucus carota) is an important root vegetable, and usually used for juice production, and there is a steady increase in carrot juice consumption (Schieber et al., 2001). In the juice industry, thousands of tons of carrot pomace are produced after the juice extraction. Carrot pomace rich in insoluble fiber-rich fraction (56.3 g/100 g of pomace), which was mainly composed of pectic polysaccharides, hemicellulose, and cellulose. This insoluble fiber-rich fraction was found to have desirable physicochemical properties such as high water- and oil-holding capacities, cation-exchange capacity, glucose-adsorbing ability, and amylase inhibition activity (Chau et al., 2004a). Carrot pomace has the highest percentage of soluble fiber when compared with apple, cabbage, strawberry, black currant, and chokeberry pomace (Chantaro et al., 2008; Nawirska and Uklanska, 2008).

Chau et al. (2004b) found that the inclusion of pomace fiber in diet effectively decreased the concentrations of serum triacylglycerol, serum total cholesterol, and liver cholesterol, and increased the concentrations of fecal total lipid, fecal cholesterol, and fecal bile acids, and showing
pronounced hypolipidemic and hypocholesterolemic effects.

The objectives of this study are to investigate the hypocholesterolemic effects of carrot pomace powder in albino rats, furthermore explore the possibility of recycling the waste by-product of food processing to produce a high fiber rich product.

Materials and methods

Materials

Fresh carrot pomace (Daucus carota) was obtained from local juice extraction shops, and then dried in an air oven at 45°C for 48 h. The dried pomace was ground in a Multi Mill apparatus and passed through a 0.5-mm mesh sieve to obtain a fine carrot pomace powder.

Chemical composition

The determination of moisture, crude protein, total lipid, ash and crude fibers were done, nitrogen free extract was calculated by difference, deducing the percentage of ash, crude protein, total lipid and crude fibers from 100 according to AOAC (2000).

Biological effects of carrot pomace powder

Experimental animals and diets

Thirty two Spraque-Dawley male albino rats weighing 110±10 g were obtained from the laboratory animal house, National Research Center. The animals were housed individually in stainless steel cages in a controlled environment (25±2°C, 50-60% relative humidity and 12-hour light-dark cycle). The animals were fed ad libitum with a basal diet and water for two weeks, and were then randomly assigned to 4 groups (8 rats each) as follows:

Group 1 (negative control): received basal diet consisting of starch 65%, casein 10%, corn oil 10%, salt mixture 4%, vitamins mixture 1% and cellulose 10% (AOAC, 2000).

Group 2 (positive control): received hypercholesterolemia-induced diet (high fat diet) which prepared as basal diet preparation, except that the 10% corn oil portion was replaced with 10% sheep fat and it was supplemented with 1% cholesterol and 0.25% bile salts (Fukushima et al., 1997).

Group 3: received 80% high fat diet plus 20% carrot pomace powder.

Group 4: received 90% high fat diet plus 10% carrot pomace powder.

Experimental design

During the experimental period (6 weeks), water and diets were available ad libitum. At the end of the experiment, all the animals were sacrificed by cervical decapitation. Blood samples were collected in two tubes. The first one (0.5 ml blood) was used for the determination of blood hemoglobin, red blood cells (RBCs), white blood cells (WBCs) and hematocrit (HCT), the 2nd heparinized tube was centrifuged at 2500 rpm at 37°C for 15 min to separate the plasma which was kept in the deep freezer for the subsequent investigation. Also, body weight, food consumption were recorded day after day.

Biochemical analysis

Lipid profile

Plasma total lipid (TL), triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were determined according to Knight et al. (1972), Fossati and Prencipe (1982), Allain et al. (1974), Levy (1981) and Burstein (1970), respectively. Atherogenic Index (AI) was calculated according to Lee and Niemann (1996) using following equation:

\[
\text{Atherogenic Index (AI)} = \frac{\text{Total Cholesterol} - \text{HDL-C}}{\text{HDL-C}}
\]

Determination of AST, ALT and ALP activities (liver functions)

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured colorimetrically at 340 nm in plasma according to the method described by Reitman and Frankel (1957). Alkaline phosphatase (ALP) activity in plasma was determined colorimetrically at 405 nm according to the method of Rec (1972).

Determination of urea and creatinine (kidney functions)

For kidney functions urea in plasma was determined colorimetrically at 578 nm according to the methods described by Patton and Crouch (1977) and the determination of plasma creatinine content was carried out colorimetrically at 510 nm according to the methods described by Faulkner and King (1976).

Determination of glucose

Plasma glucose level was determined colorimetrically at 510 nm according to Trinder (1969).

Determination of total protein and albumin

Plasma total protein and plasma albumin were determined colorimetrically according to the methods described by Henry (1976) and Doumas and Peters (1997) respectively.

Blood picture

Blood hemoglobin

The concentration of blood hemoglobin was determined colorimetrically at 546 nm according to the method of...
Red blood cells (RBCs) and White blood cells (WBCs): Red blood cells (RBCs) count and White blood cells (WBCs) count were measured according to the method of Natt and Herrick (1952).

Hematocrit (HCT): Hematocrit (HCT) was determined according to the method of Campbell (1995). Hematocrit is the percent volume of whole blood occupied by red blood cells and is determined by centrifuging blood in special (hematocrit) capillary tubes.

Statistical analysis: Statistical analysis (standard deviation “SD” and standard error “SE”) was carried out according to Fisher (1970). LSD (Least significant difference) test was used to compare the significant differences between means of treatment (Waller and Duncan, 1969). The statistical package for social science SPSS (1999) program version was used for all analysis.

Results

Chemical composition: The chemical composition of carrot pomace powder was shown in Tab. 1. The crude fiber content was (11.80%) dry weight basis, the ash content was 6.24%, crude protein 6.86 while total lipid was 1.48% dry weight basis.

Biological effects of carrot pomace powder: Tab. 2 illustrates the body weight gain (BWG), food intake and feed efficiency ratio (FER) at the end of the study. The results showed that the addition of CaPP with 10% level, decreased (BWG) (1.8±0.07), while the addition of CaPP with 20% level was highly significantly decreased (1.0±0.07) than the positive control (2.4±0.06).

It was noticed that, there was no significant changes in food intake among carrot pomace at the level of 20% (16.8±0.38), 10% (16.7±0.30) and negative control (18.0±0.60), but there was a significant decrease comparing with the positive control (20.1±0.33).

Also the feed efficiency ratio (FER) of the 20% CaPP group recorded a significant decrease (0.06±0.00) compared with positive control group (0.12±0.00) while there were no significant changes in 10% CaPP group.

On other hand 20% CaPP group showed FER value around the negative control (0.06±0.00 and 0.07±0.00) respectively.

Rats fed on hypercholesterolemia-induced diet developed hypercholesterolemia mark by significant increase in plasma total lipid, triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL-C), and atherogenic Index (AI) compared with negative control rats, while high density lipoprotein cholesterol (HDL-C) showed significant decrease (Tab. 3).

It is obvious that the intake of CaPP with different concentrations significantly alleviated the total lipid compared with positive control (1252±36.5), but with no significant changes between them (10% and 20%), (609±19.8) and (569±22) respectively.

Total cholesterol recorded high significant decrease in the different concentration of CaPP (10% and 20%) (193±4.15) and (183±4.3) respectively compared with the positive control group (388.9±11.8) while there were non significant changes with the negative control group (199±6.2).

It can be noticed that the intake of CaPP with level 20% recorded some decrease in the total cholesterol than group with 10% and the negative group. In the same table, the triglycerides showed high significant decrease in group 3 (88.9±3.6.8) and group 4 (109±3.53) compared with the positive control (395±17.2), but the decrease in triglycerides of group 3 was non significant as compared with the negative control group (124±7.6).

Data in Tab. 3 demonstrates that the increase in LDL-C was improved by supplementation with CaPP with different concentrations to hypercholesterolemic rats. The highest decrease in LDL-C was observed in group fed with 20% CaPP (114±4.05) compared with positive control (324±11.5) and negative control (140±3.4), while the 10% level of CaPP (121±3.92) was non significant compared to negative control.
diet with 20% CaPP (12.8±0.42) compared with negative control (11.2±0.55) and positive control (9.3±0.55) while the tested diet with level 10% CaPP (11.8±0.4) was non significant with negative control. Also the red blood cells (RBCs) recorded no significant changes among the negative control (5.2±0.17) and the group fed with 20% CaPP (5.1±0.15) and 10% CaPP (4.7±0.16), while recorded a significant increase in RBCs compared with the positive control group (3.6±0.18).

Tab. 4 also showed the Hematocrit Value (HCT %). The CaPP groups with 20% & 10% showed no significant change compared with negative control (39.4±1.36, 35.5±1.39 and 36.9±2.02) respectively, while they showed a significant increase around the positive control (29.1±1.03).

The white blood cells (WBCs) count represented in the same table. The positive control (3.3±0.24) and the CaPP group with 10% (4.9±0.99) showed significant decreases compared with negative control (9.6±0.84). On the other hand the value of WBCs count of CaPP group with 20% (7.1±1.42) recorded non significant changes with negative control.

Tab. 5 illustrates the liver functions in the four groups. The aspartate aminotransferase (AST) showed a high significant decrease in group 3 and 4 (27.8±1.99 and 27.3±1.44) respectively compared with positive control (93.1±6.42), but there were non significant changes with the negative control group (28.4±2.32).
Furthermore, alanine aminotransferase (ALT) was represented in the obvious table. The tested groups recorded non significant changes with the negative control. Negative control was (25.6±2.25), CaPP 20% was (24±2.23) and CaPP 10% (24.8±2.28), on the other hand there were a high significant decrease compared with the positive control group (86±4.04). The same results in alkaline phosphatase (ALP) which showed a very highly significant decrease in the CaPP groups 10% and 20% (90.4±6.18 and 84.5±6.36) respectively compared with positive control.

Tab. 6. Plasma urea and creatinine (mg/dL) of the experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-1 Negative Control</td>
<td>26.6±3.1 a</td>
<td>0.9±0.08 b</td>
</tr>
<tr>
<td>G-2 Positive Control</td>
<td>12.9±1.73 b</td>
<td>1.2±0.07 a</td>
</tr>
<tr>
<td>G-3 20% CaPP</td>
<td>26.9±2.96 a</td>
<td>0.9±0.06 b</td>
</tr>
<tr>
<td>G-4 10% CaPP</td>
<td>19.5±2.92 ab</td>
<td>1.2±0.05 a</td>
</tr>
</tbody>
</table>

All values represented as mean ±S.E. Means with different letters are significantly different (p<0.05). * CaPP (carrot pomace powder)

Tab. 7. Plasma protein, albumin (g/dL) and plasma glucose (mg/dL) of the experimental rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameters</th>
<th>Total Protein (g/dL)</th>
<th>Albumin (g/dL)</th>
<th>Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1 Negative Control</td>
<td>7±0.26 a</td>
<td>4±0.28 ab</td>
<td>87±6.67 b</td>
<td></td>
</tr>
<tr>
<td>G-2 Positive Control</td>
<td>4.2±0.28 c</td>
<td>2.5±0.24 c</td>
<td>105±3.95 a</td>
<td></td>
</tr>
<tr>
<td>G-3 20% CaPP</td>
<td>7.5±0.2 a</td>
<td>4.3±0.17 a</td>
<td>87.5±3.52 b</td>
<td></td>
</tr>
<tr>
<td>G-4 10% CaPP</td>
<td>6±0.23 b</td>
<td>3.5±0.18 b</td>
<td>90.5±3.16 b</td>
<td></td>
</tr>
</tbody>
</table>

All values represented as mean ±S.E. Means with different letters are significantly different (p<0.05). * CaPP (carrot pomace powder)

Tab. 8. Relative organs weight (g) of the experimental rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Organs</th>
<th>Liver</th>
<th>Heart</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1 Negative Control</td>
<td>9.1±0.27 d</td>
<td>1.3±0.06 a</td>
<td>2.2±0.05 b</td>
<td>1.1±0.05 a</td>
<td></td>
</tr>
<tr>
<td>G-2 Positive Control</td>
<td>20.0±0.85 a</td>
<td>1.4±0.05 a</td>
<td>2.7±0.06 a</td>
<td>1.3±0.06 a</td>
<td></td>
</tr>
<tr>
<td>G-3 20% CaPP</td>
<td>11.6±0.80 c</td>
<td>1.3±0.07 a</td>
<td>2.3±0.05 b</td>
<td>1.2±0.05 a</td>
<td></td>
</tr>
<tr>
<td>G-4 10% CaPP</td>
<td>15.0±0.63 b</td>
<td>1.4±0.05 a</td>
<td>2.5±0.07 a</td>
<td>1.2±0.05 a</td>
<td></td>
</tr>
</tbody>
</table>

All values represented as mean ±S.E. Means with different letters are significantly different (p<0.05). * CaPP (carrot pomace powder)

Data in Tab. 6. represents the results of kidney function tests. Urea records an increase in CaPP with about 20% level (26.9±2.96) but this increase was non significant with the negative control group (26.6±3.1), on the other side CaPP with 10% level (19.5±2.92) was non significant with the positive control group (12.9±1.73). Also the creatinine concentration showed in the same table recorded a significant decrease in 20% CaPP group (0.9±0.06) compared with the positive control (1.2±0.07) while the 10% CaPP group (1.2±0.05) showed non significant changes with the positive control.

The results presented in Tab. 7. indicate the hypercholesterolemia effects on blood glucose and plasma total protein and albumin. Total protein recorded a non significant changes between negative control (7±0.26) and 20% CaPP group (7.5±0.2), while the 10% CaPP showed a significant decrease (6±0.23) which was also significant compared with the positive control (4.2±0.28) which recorded the lowest value.

Albumin was significantly decreased in 10% CaPP group (3.5±0.18) and positive control (2.5±0.24) compared with the 20% CaPP group (4.3±0.17) which was non significant compared with the negative control (4±0.28). In the same table, glucose records an increase in positive control group (105±3.95), this increase in glucose level improved by supplementation with 20% CaPP (87.5±3.52) and 10% CaPP (90.5±3.16) this improve reached to the normal level of glucose in negative control group (87±6.67).

Data in Tab. 8. illustrate the organs weight of the experimental rats, supplementations decreased the liver weight in CaPP with 10% (15.0±0.63) and the 20% level (11.6±0.80) compared with positive control group (20.0±0.85), on the other hand the negative control group recorded the lowest liver weight (9.1±0.27). Kidney has non significant changes in weight between the 10% CaPP group (2.5±0.07) and positive control (2.7±0.06), also the 20% CaPP group (2.3±0.05) showed a value around the negative control (2.2±0.05). Finally, heart and spleen weights showed non significant changes in all groups.

Discussion

Chemical composition of CaPP was shown that Capp is rich in fibers (11.8%) dry weight, results was agree with Bao and Chang (1994) who found that the carrot pomace is rich in fiber more than carrot peels and more that some agriculture by-products such as pear, orange, peach. Also these results are in the same line with Chau et al. (2004a) who found that carrot pomace was rich in dietary fiber and low in protein whereas, the values reported by Holland et al. (1991) for most of these parameters are different i.e. moisture (88.8%), protein (0.7 %), fat (0.5%), crude fiber (2.4%).
The increase in BWG, FI and FER for positive control which fed on hypercholesterolemic diet may be due to the presence of animal fat and cholesterol used to increase feeding. Also results revealed that the reduction in BWG of the rats fed on diet containing 20% CaPP indicate muscle tissue wasting this decrease may due to high content of fiber, these results were agreed with Parveen et al. (2000) who reported that the fiber content of diet reduce calories and losses weight.

However Chau et al. (2004b) was on an opposite side, they found that consumption of water-insoluble fiber rich fraction didn’t affect the weights of hamsters. Also Nicolle et al. (2003) were on contrary line with our study who found no significant changes on body weight gain and food intake in the groups feed on carrot diet with the control group.

The addition of carrot in the diet with 10, 20% concentrations lead to decrease in total lipid, total cholesterol, triglycerides and LDL-C but resulting increase of HDL-C. Our data was in the same line with Nicolle et al. (2003) who found carrot consumption exerts a moderate lowering cholesterol effect (12% decreases). A significant 11% reduction of cholesterol has been observed in human subjects by Robertson et al. (1979).

Hsu et al. (2006) recorded significant decrease in concentration of serum triglyceride, serum total cholesterol of hamsters fed on diet containing insoluble fiber-rich fractions prepared from carrot pomace. Whereas an absence of effect was reported by Wisker et al. (1994).

The improvement in lipid profile of blood could be referred to a multi factors besides on the role of amino acids of protein, dietary fibers and antioxidants may play a good part in this action. Beneficial treatment of CaPP showed that the dietary fibers are having the potential to lower the levels of total cholesterol and LDL-c in blood. Absorption of bile salts by soluble dietary fiber (SDF) results in changes in cholesterol metabolism, loss of cholesterol, unavailability of bile salts in the intestine for micelle formation, which inhibits lipid fractions absorption, increased fecal bulk dilutes bile acids in the lower intestinal tract, and short chain fatty acids produced especially the propionate, which has been proposed to inhibit hepatic cholesterol synthesis (Tharanathan and Mahadevamma, 2003).

The improvement in blood picture data was noticed especially in hemoglobin which recorded a significant increase in 20% CaPP than control group also hematocrit was in significant increase in 10%, 20% CaPP compared with positive control, this increase in blood picture may due to the high presence of iron. These data were corresponding with Sharma et al. (2012) who found that carrot are a good source of minerals like Ca, P, Fe and Mg. Also Gopalan et al. (1991) reported the chemical constituents of carrot as moisture (86%), protein (0.9%), crude fiber (1.2%) and Fe (2.2 mg/100g), Ca (80 mg/100g) and P (53 mg/100g).

The previous results indicated that the rats fed on hypercholesterolemic diet showed increased liver enzymes (AST, ALT and Alkaline phosphatase). The liver is a central organ for many physiological and biochemical process necessary for maintenance of life (Souba and Wilmore, 1983). Morphological alterations that occur in the liver affect many metabolic processes in the organism. Peroxide formation induced by hypercholesterolemia (Sudhahar et al., 2007) result in the release of some enzymes by interacting with cellular structure and function. Thus, the serum activities of cellular enzymes such as transaminases, alkaline phosphatase, and lactate dehydrogenase do increase. With the increase in cellular membrane permeability, intracellular fluid transfers onto intercellular space, resulting in muscle and liver cell degeneration.

Rats fed on 10 and 20% CaPP supplemented to hypercholesterolemic diet showed improved liver functions. AST and ALT levels act as indicators of liver functions, hence, restoration of normal levels of these enzymes indicates normal functions of liver. The reduction of AST and ALT close to their normal levels due to consumption of CaPP (Quanhong et al., 2005). The present study also revealed that the ALP activity was increased when the liver functions abnormally (Rashad and Moharib, 2008), thus the study of liver ALP was done in the present study to find out the effect of these dietary fiber (CaPP) on liver ALP.

Results showed that CaPP with 10 and 20% had a lowering effect on the activity of ALP in serum of hypercholesterolemic rats compared to the positive control, but this decrease was not significant with the negative control group. This effect is mainly related to the presence of natural soluble and insoluble dietary fiber (Rashad and Moharib, 2003).

The result of the present study also declared that CaPP especially at 20% level has a significant improvement effect on the kidney functions represented in urea and creatinine. This could be explained as consumption of food rich in dietary fibers stimulates the extrarenal route of nitrogen excretion. Younes et al. (1998) found that indigestible carbohydrate/dietary fibers increased cecal weight and cecal blood flow, leading to accelerated diffusion of blood urea into the cecal lumen (by threefold), urea lysis to ammonia and protein synthesis by the microflora, and increased fecal excretion of nitrogen. Thus, reduce the role of kidney in the excretion of nitrogen and reduce blood urea concentration.

Total protein and albumin were markedly increased with addition of 10%, 20% CaPP especially with 20%, these results were on a opposite side to Eggum (1992) who reported that dietary fiber had a negative influence on digestion and assimilation of proteins. Also results show a markedly decrease of glucose on hypercholesterolemic rats fed on 10 and 20% CaPP. These results were confirmed by Chau et al. (2004a) who found that fiber has functional properties and in vitro hypoglycemic effects. Rodríguez...
et al. (2006) declared that glucose of diabetic patients decreased by having diets rich in fiber. These results given are in a good agreement with Singh et al. (2005) who found that feeding of PoPP at 5% and 10% to diabetic rats significantly decreased their blood glucose level.

It could be noticed from previous results that hypercholesterolemia increased organs weight especially liver and kidney compared with negative control, while their spleen and heart had no significant changes in their weight. However, 10% and 20% CaPP supplemented to hypercholesterolemic treated groups had a significantly decreased in liver and kidney weights.

Conclusions

Finally the present results clearly refer to possibility using carrot pomace powder as hypocholesterolemic agent. In addition, CaPP also serves to improve the lipid profile (cholesterol, total lipid, triglycerides, LDL-C and HDL-C) and blood picture and to reduce the blood glucose level in hypercholesterolemic rats and could use in obese people for body loss.

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