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Physalis peruviana pomace suppresses high-cholesterol diet-induced hypercholesterolemia in rats

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RESUMEN

El orujo de *Physalis peruviana* suprime la hipercolesterolemia inducida por una dieta rica en colesterol en ratas.

Physalis peruviana (aguaymanto) es un fruto prometedor que puede ser parte de diferentes alimentos funcionales. No hay datos disponibles sobre el efecto de la administración del orujo de aguaymanto sobre diferentes aspectos del perfil de lipídos plasmáticos en animales de experimentación. De acuerdo con la composición química del orujo de la fruta que incluye altos niveles de compuestos bioactivos, se demostró la hipótesis de que la alimentación con orujo de *Physalis peruviana* puede tener efectos saludables y sobre la hipercolesterolemia en ratas alimentadas con una dieta alta en colesterol (HCD). Por tanto, el objetivo de este estudio fue investigar el efecto de una alimentación con orujo de *Physalis peruviana*so sobre la hipercolesterolemia analizando los cambios del perfil lipídico en ratas alimentadas con una dieta alta en colesterol (HCD). Se determinó la composición química, el perfil lipídico (ácidos grasos, tocoferoles y esteroles) y contenido fenólico del orujo de aguaymanto. En términos generales, las ratas alimentadas con orujo de aguaymanto mostraron niveles más bajos de colesterol total (TC), triglicéridos totales (TAG) y lipoproteínas de baja densidad totales, así como superiores niveles de lipoproteínas de alta densidad (HDL) en comparación con los animales alimentados con HCD y con una dieta libre de colesterol (CFD). El examen histológico del hígado y de los riñones fue también realizado. Los resultados demostraron que el consumo de orujo de aguaymanto proporciona efectos beneficiosos generales invirtiendo los cambios perjudiciales asociados a una dieta HCD.

PALABRAS CLAVE: Colesterol – Colesterol-HDL – Colesterol LDL – Hipercolesterolemia – Perfil lipídico – Ratas.

SUMMARY

***Physalis peruviana* pomace suppresses high-cholesterol diet-induced hypercholesterolemia in rats.**

Physalis peruviana (goldenberry) is a promising fruits that can be an ingredient in several functional foods. No reports are available on the effect of the administration of goldenberry pomace on different aspects of the plasma lipid profile in experimental animals. According to the chemical composition of the fruit pomace which includes high levels of bioactive compounds, the hypothesis was that feeding *Physalis peruviana* pomace may have health-promoting and hypercholesterolemic impacts on rats fed a high cholesterol diet (HCD). Therefore, the objective of this study was to investigate the effect of feeding goldenberry pomace on hypercholesterolemia by analyzing the changes in lipid profiles in HCD fed rats. The chemical composition, lipid profiles (fatty acids, tocopherols and sterols) and phenolic contents of the fruit pomace were determined. Generally, rats fed the fruit pomace showed lower levels of total cholesterol (TC), total triacylglycerol (TAG) and total low density lipoprotein (LDL) cholesterol as well as higher levels of high density lipoprotein (HDL) cholesterol in comparison with animals fed HCD and cholesterol free diets (CFD). Histological examinations of the liver and kidney were also studied. The results demonstrated that goldenberry pomace consumption provides overall beneficial effects on reversing HCD associated detrimental changes.

KEY-WORDS: Cholesterol – Cholesterol-HDL – Cholesterol-LDL – Hypercholesterolemia Lipid profile – Physalis peruviana – Pomace – Rats

1. INTRODUCTION

Berries have been shown to provide health benefits because of their high antioxidants, vitamins, minerals and fiber (Zhao, 2007). Goldenberries or cape gooseberries (*Physalis peruviana* L., Solanaceae) are short-lived perennials. The fruit, with an approximate weight of 4-5 g is protected by an accrescent calyx and covered with a bright yellow peel (Mayorga *et al*., 2001). The Goldenberry has been grown in Egypt, South Africa, India, New Zealand, Australia and Great Britain (Ramadan and Mörsel, 2003; 2004). International markets exist for many exotic fruits and recently the processing of tropical fruits has begun in many countries (Ramadan and Mörsel, 2007). In 2005, there were more than 1.8 million acres of berry crops worldwide including 966 acres of gooseberries (Strik, 2007). The single plant may yield 300 fruits and carefully tended plants can provide 20 to 33 tons/hectare. The fruit has been used as a good source of provitamin A, minerals, vitamin C and vitamin B-complex. Goldenberry juice yield is about 70% of the berry weight (Ramadan and Mörsel, 2007). The juice is rich in fat-soluble bioactive compounds (tocopherols and phytosterols) and could be a novel source of functional drinks (Ramadan, 2011). Fruit pomace

(seeds and skins) represents the waste generated during juice processing (*ca.* 27.4% of fruit weight). The pomace contains 19.3% oil, 17.8% protein, 3.10% ash, 28.7% crude fiber and 24.5% carbohydrates (Ramdan and Mörsel, 2009). The enzymatic hydrolysis of pomace followed by solvent extraction reduced the extraction time and enhanced oil extractability up to *ca*. 7.60% (Ramadan *et al*., 2008b).

Blood cholesterol is of great importance because blood total cholesterol (TC) and low-density lipoprotein (LDL) correlate strongly with coronary heart disease (CHD). Cholesterol homeostasis is maintained by a complex mechanism of sterol absorption, anabolism, catabolism and excretion (Chen *et al*., 2011, El-Anany and Ali, 2012). Current research initiatives in nutrition indicate that fruits, vegetables, grains and oilseeds are receiving increased attention as essential components of the human diet (Ramadam *et al*., 2011). The contribution of bioactive compounds (phenolic compounds, vitamins, polyunsaturated fatty acids (PUFA) and fat-soluble bioactives) in biological systems and many degenerative diseases and CHD have been studied. Hypercholesterolemia is a major risk factor for the development of atherosclerosis and related cardiovascular diseases (Deepa and Varalakshmi, 2005). A high-cholesterol diet (HCD) is a major environmental contributor to an unbalanced lipoprotein metabolism. It is associated with an increased prevalence of atherosclerosis which is the major source of morbidity and mortality in the developed world (Stocker and Keaney, 2004). Previous research has indicated a positive correlation between serum cholesterol level and the risk of CHD (Leys *et al*., 2002). Despite the fact that there are drugs available clinically for treating hypercholesterolemia, the consumption of functional foods in controlling serum cholesterol levels and risk of CHD has gained enormous global acceptance over the years. The concentration of cholesterol can be regulated by cholesterol biosynthesis, removal of cholesterol from the circulation, absorption of dietary cholesterol and excretion of cholesterol *via* bile and feces (Vijaimohan *et al*., 2006). In this study, we tested the hypothesis that the consumption of goldenberry pomace provides an overall improvement to alleviate the detrimental outcomes associated with HCD in the liver and the cardiovascular systems. The goal was to study the impact of feeding goldenberry pomace on the lipid profile of rats upon feeding HCD with the objective of promoting their use in functional foods and neutraceuticals.

2. MATERIALS AND METHODS

2.1. Materials

Ripe *Physalis peruviana* fruits were obtained from local growers in Zagazig (Sharkiah, Egypt). Intact fruits were carefully selected according to their degree of ripeness measured by fruit color (bright orange), pH value of the pulp (3.86) and total titratable acidity (0.92%). Caffeic acid was from Fluka AG (Buchs, Switzerland). Standards used for sterol (ST) characterization were purchased from Supelco (Bellefonte, PA, USA). Standards used for vitamin E (α-, β-, γ- and δ-tocopherol) were purchased from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation of berry pomace

Whole berries were blended in a warring blender (Moulinex Ovatio 3, France) for 5 minutes. To remove the seeds and skin residues (fruit pomace), the juice was filtered through cheesecloth. The fruit pomace was freeze-dried (Alpha 1-5, Martin Christ, Osterode am Harz, Germany) till the moisture content reached *ca.* 15%. The dried pomace was ground (Analysenmühle A10, Janke & Kunkel GmbH, Germany), screened to 0.125 mm particle size and kept at 4 °C until analysis.

2.2.2. Chemical composition of pomace

Pomace samples were analyzed by the Standard AOAC procedures (1995) for moisture, ash and fiber contents. Nitrogen was determined by micro-Kjeldahl and a factor of 6.25 was used for protein conversion. From a part of the raw finely ground fruit byproducts, the oil was extracted with *n*-hexane in a Soxhlet extractor for 8 h. All the analytical determinations were done in triplicate.

2.2.3. Analysis of recovered lipids from pomace

Fatty acids, ST and tocopherols were determined in the lipid from pomace according to Ramadan *et al*. (2010).

2.2.4. Gas chromatography (GC) analysis of fatty acid methyl esters (FAME)

Fatty acids were transesterified into FAME using N-trimethylsulfoniumhydroxide (Macherey-Nagel, Düren, Germany). FAME were identified on a Shimadzu GC-14A equipped with a flame ionization detector (FID) and C-R4AX chromatopac integrator (Kyoto, Japan). The flow rate of the carrier gas helium was 0.6 mL/min and the split value with a ratio of 1:40. A sample of 1 µL was injected into a 30 m × 0.25 mm × 0.2 µm film thickness Supelco SP™-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperature was set at 250 °C. The initial column temperature was 100 °C programmed at 5 °C/min until 175 °C and kept for10 min at 175 °C, then 8 °C min^{-1} until 220 °C and kept for 10 min at 220 °C. A comparison was made between the retention times of the samples with those of an authentic standard mixture (Sigma, St. Louis, MO, USA; 99% purity

specific for GLC), run in the same column under the same conditions in order to facilitate identification.

2.2.5. Gas chromatography (GC) analysis of sterols (ST)

Separation of ST was performed after saponification of the oil sample without derivatization according to Ramadan and Mörsel (2003). Oil samples (250 mg) were refluxed with a 5 mL ethanolic potassium hydroxide solution (6%, w/v) and a few anti-bumping granules for 60 min. The unsaponifiables were first extracted 3-times with 10 mL of petroleum ether, the extracts were combined and washed 3-times with 10 mL of neutral ethanol/water (1:1, v/v) and then dried overnight with anhydrous sodium sulphate. The extract was evaporated in a rotary evaporator at 25 °C under reduced pressure, and then ether was completely evaporated under nitrogen. GLC analyses of unsaponifiable residues were carried out using a Mega Series (HRGC 5160, Carlo Erba Strumentazione; Milan, Italy) equipped with FID. The following parameters were performed: DB 5 column (J&W scientific; Falsom, CA, USA) packed with 5% phenylmethylpolysiloxan, 30 m length, 0.25 mm i.d., 1.0 µm film thickness; carrier gas (helium) flow 38 $mLmin^{-1}$ (split-splitless injection was used). Detector and injector were set at 280 °C. The oven temperature was kept constant at 310 °C and the injected volume was 2 µL. The repeatability of the analytical procedure was tested and the relative standard deviation of three repeated analyses of a single sample was <5%. Quantitative analyses were performed with a Shimadzu (C-R6A Chromatopac; Kyoto, Japan) integrator.

2.2.6. Normal phase high performance liquid chromatography (NP-HPLC) separation, identification and quantification of tocopherols

Procedure. NP-HPLC was selected to avoid extra sample treatment (e.g., saponification) according to Ramadan and Mörsel (2003). The analysis was performed with a solvent delivery LC-9A HPLC (Shimadzu, Kyoto, Japan). The chromatographic system included a model 87.00 variable wavelength detector and a 250 × 4 mm i.d. LiChrospher-Si 60, 5 µm, column (Knauer, Berlin, Germany). The separation of tocopherol isomers was based on isocratic elution when the solvent flow rate was maintained at 1 mL/min at a column back-pressure of about 65-70 bar. The solvent system selected for elution was isooctane/ethyl acetate (96:4, v/v) with detection at 295 nm. Twenty µL of the diluted solution of total lipids (TL) in the mobile phase were directly injected into the HPLC column. Tocopherol isomers were identified by comparing their retention times with those of authentic standards.

Preparation of standard curves. Standard solutions were prepared by serial dilution to a concentration of approximately 5 mg mL^{-1} of each tocopherol isomer. Standard solutions were prepared from a stock solution which was stored in the dark at –20 °C. Twenty µL were injected and peaks areas were determined to generate standard curve data.

Quantification. All quantitation was by peak area using a Shimadzu C-R6A chromatopac integrator (Kyoto, Japan). Standard curves (concentration versus peak area) were calculated from six concentration levels by linear regression. Based on the established chromatographic conditions, repeated injections of different concentrations of the standard tocopherols were made 3-times onto the HPLC system. Injections in triplicate were made at each concentration for both standards and samples. All work was carried out under subdued light conditions. All the experiments were repeated at least three times when the variation in any one was routinely less than 5%.

2.2.7. Phenolic compounds in fruit pomace

The total phenol content was measured by the Folin-Ciocalteau reagent using caffeic acid as standard. One millilitre of pomace 50% methanolic extract was mixed with 5 mL of Folin-Ciocalteau reagent (previously diluted 30-fold with distilled water) and 15 mL of sodium bicarbonate (20 g 100 mL^{-1}), then the mixture was diluted to 100 mL with distilled water. The solution was kept in the dark at room temperature for 2 h then the absorbance was measured at 760 nm using a Shimadzu UV-260 spectrophotometer (Kyoto, Japan). The mean value of total phenolic contents was obtained from triplicate experiments.

2.2.8. Animal experiment

Experimental diets. Fruit pomace was incorporated into the experimental diets and the full composition of experimental diets is detailed in Table 1. The basal diet included wheat starch, casein, cellulose, and mineral and vitamin mixtures. Table 2 presents the composition of minerals and vitamins in the diet used in this study. The work was carried out at the Agricultural Biochemistry Department, Faculty of Agriculture, Zagazig University. To study the effects of two concentrations of goldenberry pomace (10% and 30%) on the lipid profile of albino rats, twenty male albino rats (weighing between 120 and 140 g) were used in this study. The experiment lasted for 60 days and the animals were divided into 4 groups each containing 5 rats. Animal group numbers 3 and 4 represent control groups where group 4 was fed a basal diet as the negative (–) control while control group (3) received HCD till the end of the experiment to serve as the positive (+) control. Groups 1 and 2 were allowed to feed hypercholesterolemic diets (1% cholesterol + 0.25% colic acid) supplemented with golden berry pomace (10% for group 1; and 30% for group 2) as mentioned in Table 1. The rats were housed in cages with a screen bottom in a controlled environment with 12 h light and 12 h dark cycles. Diets and water were available over the 60-day period and the rats were weighed every week. All

Table 1
Chemical composition (g kg^{-1}) and calculated analysis of experimental diets

	HCD			CFD
	Group 1 (10% Pomace)	**Group 2 (30% Pomace)**	**Group 3 (positive control)**	**Group 4 (negative control)**
Casein	150	150	150	150
Starch	637.5	437.5	737.5	747.5
Fruit pomace	100	300	–	–
Salt mixture	40	40	40	40
Vitamin mixture	10	10	10	10
Cellulose	50	50	50	50
Colic acid	2.5	2.5	2.5	2.5
Cholesterol	10	10	10	–

Table 2
Composition of vitamin and mineral mixture in diet*

Vitamins	Quantity	Minerals	Quantity
Vitamin A	2000 iu	NaCl	0.5 %
Vitamin D	200 iu	KI	0.013 %
Vitamin E	75 iu	K_2HPO_4	1.62 %
Vitamin K	0.5 mg	$MgSO_4$	0.325%
Inositol	10 mg	$CaCO_3$	1.5%
Niacin	4.0 mg	$CaHPO_4$	0.30%
Ca pantothenate	4.0 mg	$FeSO_4$	0.125%
Riboflavin	0.8 mg	$CuSO_4$	0.0015%
Thiamin HCL	0.5 mg	$MnSO_4$	0.011%
Pyridoxine	0.5 mg	$ZnSO_4$	0.00916%
Folic acid	0.2 mg		
Biotin	0.04 mg		
Vitamin B12	0.003 mg		
Choline chloride	200 mg		
p-amino benzoic acid	10 mg		

*starch was added to make 1000 mg

groups were fed the basal diet for 10 days as an adaptation period.

Blood sampling and analysis. Blood samples were collected after 60 days from the start of the experiment for analysis. Total cholesterol was analyzed according to Richmond (1973). Blood samples were collected at the end of the study by sacrificing all the groups by decapitation after overnight fast and part of the blood samples were collected into tubes and then centrifuged at 3000 rpm for 15 minutes to obtain plasma, which was kept frozen until analysis. Total lipids (TL) were determined by the reaction of TL with a sulfophosphovanillic mixture according to Coudon and Bouige (1973). According to Demacker *et al*. (1984) LDL–cholesterol was determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by the polyvinyl sulfate in the presence of polyethyleneglycol monomethyl ether. Triacylglycerols were analyzed according to Fossati and Prencipe (1982).

Liver and Kidney functions assay. Transaminases SGPT, SGOT, total serum protein, serum albumin, and serum globulins were determined according to Reitman and Frankel (1957), Henry (1964), Doumas *et al*., (1971) and Reinhold (1953), respectively. Kidney functions including serum uric acid and blood urea were determined according to Patton and Crouch (1977).

Histopathological examinations. Three randomly selected male rats from each treated group were anesthetized under ether and sacrificed by cervical

dislocation at the end of the experiment period. For the histopathological examination, vital organs such as kidney and liver tissues were dissected out and examined grossly. Subsequently, the tissue specimens were taken and fixed in a 15% formalin saline for the histopathological alternations. The fixed tissues were processed by dehydration in a series of graded ethanol concentrations, cleared with xylol and embedded in paraffin blocks. Sections at 4 µm thickness were obtained and stained according to the Hematoxilen and Eosin method to further analysis in a light microscope for histopathological changes as described previously (Ratcliffe, 1982).

2.2.9. Statistical analysis

Results are presented as average ± standard deviation of the means. One way analysis of variance (ANOVA) was used to ascertain whether the dietary treatments were a source of variance related to the various lipid parameters measured. If a significant F test was noted, means were separated using critical difference. A level of $P < 0.05$ was considered significant.

3. RESULTS

3.1. Chemical composition and bioactive compounds in fruit pomace

Goldenberry pomace contained 6.6% moisture, 17.8% protein, 3.10% ash, 28.7% crude fiber and 24.5% carbohydrates (determined by difference). The *n*-hexane-extractable oil content of the starting raw byproducts was estimated to be 19.3%. According to the results shown in Table 3, ten fatty acids were detected. Fatty acid analysis gave the proportion of linoleic, oleic, palmitic and stearic as the major fatty acids. Linoleic acid was the dominating fatty acid followed by oleic acid as the second major fatty acid, where the ratio of linoleic acid to oleic acid was higher than 7:1. It is well known that lipids, rich in linoleic acid, prevent cardiovascular disorders such as coronary heart diseases, atherosclerosis and high blood pressure. The pomace oil also contains appreciable amounts of saturated normal chain fatty acids. Palmitic acid (*ca*. 8%) followed by stearic acid (*ca*. 2.6%) were the major saturated fatty acids. The four fatty acids (linoleic, oleic, palmitic and stearic) constituted *ca*. 97% of total FAME in pomace oil. Six minor fatty acids, namely palmetoleic (C16:1 *n*-7), arachidic (C20:0), γ-linolenic (C18:3*n*-6), erucic (C22:1 *n*-9) and eicosapentaenoic (C20:5n-3, EPA) were also estimated in pomace oil. The fatty acid composition and high proportions of PUFA make the goldenberry a special fruit for nutritional applications. The contents and composition of free ST and tocopherols determined in goldenberry pomace oil are shown in Table 3. The oil is mainly characterized by high levels of unsaponifiables (22.0 g kg^{-1}). Campesterol was the ST marker (*ca*. 43% of total sterols). The next major components were Δ5-avenasterol and lanosterol. These three major components represented more than 75% of total ST. Δ7-Avenasterol and β-sitosterol were presented in approximately equal amounts (*ca*. 6.0% of total ST). Phytosterols are of interest due to their antioxidant

Table 3
Levels of fatty acids and fat-soluble bioactives of goldenberry pomace lipids

Fatty acid	Pomace oil	Compound	Pomace oil
C16:0	7.95 ± 0.09		
C16:1*n*-7	0.62 ± 0.01	α-Tocopherol (g kg^{-1})	0.34± 0.01
C18:0	2.61 ± 0.03	β- Tocopherol (g kg^{-1})	2.10 ± 0.13
C18:1*n*-9	10.3 ± 0.15	γ- Tocopherol (g kg^{-1})	1.08 ± 0.11
C18:2*n*-6	77.1 ± 0.36	δ- Tocopherol (g kg^{-1})	0.85 ± 0.05
C20:0	0.87 ± 0.01		
C18:3*n*-6	0.06 ± 0.01	Ergosterol (g kg^{-1})	0.15 ± 0.01
C22:1*n*-9	0.16 ± 0.01	Campesterol (g kg^{-1})	4.70 ± 0.12
C20:5 EPA	0.15 ± 0.01	Stigmasterol (g kg^{-1})	0.28 ± 0.07
C22:6 DHA	0.18 ± 0.01	Lanosterol (g kg^{-1})	1.60 ± 0.17
Total saturated	11.4	β-Sitosterol (g kg^{-1})	1.04 ± 0.05
Total monoenes	11.0	Δ5-Avenasterol (g kg^{-1})	2.63 ± 0.18
Total dienes	77.1	Δ7-Avenasterol (g kg^{-1})	0.56 ± 0.04
Total trienes	0.06		
S/U ratio (%)[a]	12.8	Total Unsaponifiable (g kg^{-1})	22.0 ± 0.10

[a] Ratio of saturated to unsaturated fatty acids.
Values are given as means of three replicates ± standard deviation.

activity and their impact on health. Thus, STs have recently been added to vegetable oils to formulate a successful functional food (Ramadan *et al*., 2008b). Statistics regarding the qualitative and quantitative composition of tocopherols are given in Table 3. β-Tocopherol (47%) and γ-tocopherols (26%) were the major components. On the other hand, δ-tocopherol constituted only *ca*. 18.5% of the total tocopherol content followed by α-tocopherol (*ca.* 6.0%). Despite a general agreement that α-tocopherol is the most efficient antioxidant and vitamin E homologue in *vivo*, studies indicate a considerable discrepancy in its absolute and relative antioxidant effectiveness in *vitro*, especially when compared to γ-tocopherol. High amounts of tocopherols detected in the examined extracts may contribute to great stability toward oxidation of these lipids. Phenols make up a part of the "polar fraction" of vegetable oils. The total phenolics level of the fruit pomace was 323 mg kg^{-1} (CAE). To assist in characterizing phenolic compounds, absorption ranges were scanned between 200 and 400 nm. The UV spectra of methanolic solutions exhibited two absorption maxima (282 nm and 320 nm). The absorption maximum at the longer wavelength (320 nm) may be due to the presence of phenolic acids, while the absorption maximum at the shorter wavelength (280 nm) may be due to the presence of *p*-hydroxybenzoic acid and flavone/flavonol derivatives (Ramadan *et al*., 2010).

3.2. Impact of feeding pomace on the plasma lipid profile

Goldenberry has been reported to have a variety of biological activities (Ramadan and Moersel, 2009). For the hypolipidemic and antioxidant effects, scientific data on its efficacy are scarce. In the present study, we examined whether goldenberry pomace might improve the lipid profile and oxidative damage resulting from an HCD in rats.

3.2.1. Triacylglycerols (TAG) and total cholesterol (TC)

TAG in the blood tends to damage vascular endothelial cells, leading to heart disease. A high fat diet produces an increase in TAG levels due to lipoprotein lipase TAG hydrolysis, so that the accumulation in the liver becomes more evident (Feoli *et al*., 2003). In contrast, the effect of PUFA can be attributed to a reduction in the hepatic synthesis of fatty acid, which decreases the

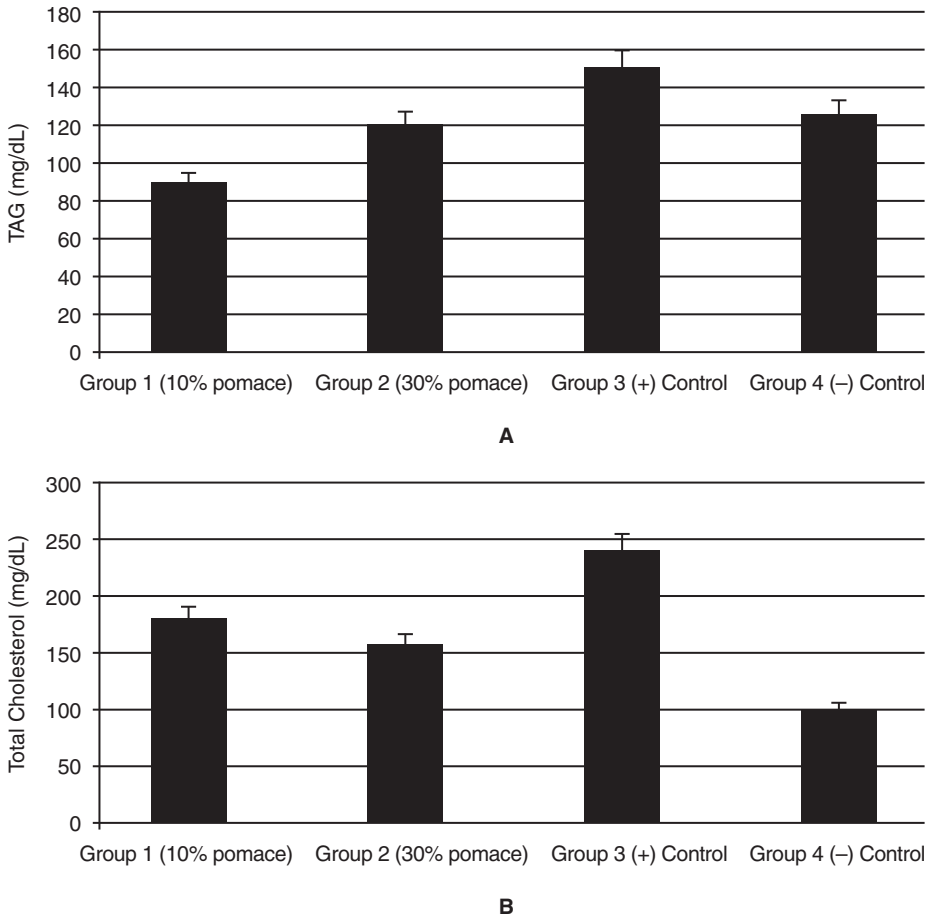

Figure 1
Impact of feeding goldenberry pomace on total triacylglycerols (A) and total cholesterol (B). Values given are the mean of three replicates and error bars show the variations of three determinations in terms of standard deviation

concentration of TAG in the liver. The data in Figure 1A show the levels of serum TAG of hyperlipidemic rats fed fruit pomace. After 60 days, the data revealed that all groups showed a decrease in serum TAG in comparison with the positive control group. Groups 1 and 2 possessed lower levels of TAG in comparison with the control groups. These effects might be due to high plasma lipoprotein lipase activity, an enzyme involved in hydrolysis of plasma VLDL triacylglycerols. After 60 days of feeding, the data show that group 4 (CFD) had the lowest serum TC followed by group 2 in comparison with the HCD group (Figure 1B). Feeding rats with fruit pomace (30%) caused a decrease in total cholesterol of 35% while group 1, which was fed 10% pomace showed a decrease of 23% compared with the positive control group.

3.2.2. High and low density lipoprotein cholesterol (HDL- and LDL-cholesterol)

In contrast to the adverse effects of an elevation in LDL, the concentration of HDL is inversely correlated with atherosclerosis development (Khera and Rader, 2010) and as an antioxidant inhibits the oxidative modification of LDL (McPherson *et al*., 2007). The inverse association between the incidence of CHD and HDL-cholesterol levels has been known. HDL is an important scavenger of surplus cholesterol transporting it from cell membranes to the liver where it is metabolized or converted into bile acids. However, the mechanism of the increase or maintenance of HDL-cholesterol is not clear. After 60 days of the feeding experiment it was generally noted that the levels of HDL increased in both groups (1 and 2) compared with the control groups (Figure 2A). The highest increase in HDL was measured in group 2 (37 mg dL^{-1}) followed by group 1 (32.5 mg dL^{-1}). HDL has been indicated as a positive factor in determining the development of atherosclerosis (Miller and Miller, 1997). Supplementation with goldenberry pomace increased the rate of HDL-cholesterol. HDL plays an important role in the transport of cholesterol from peripheral cells to the liver, and hence the elevated serum HDL–cholesterol levels through dietary goldenberry pomace are considered to be favorable since there is a negative correlation between HDL–cholesterol levels and risk of cardiovascular disease.

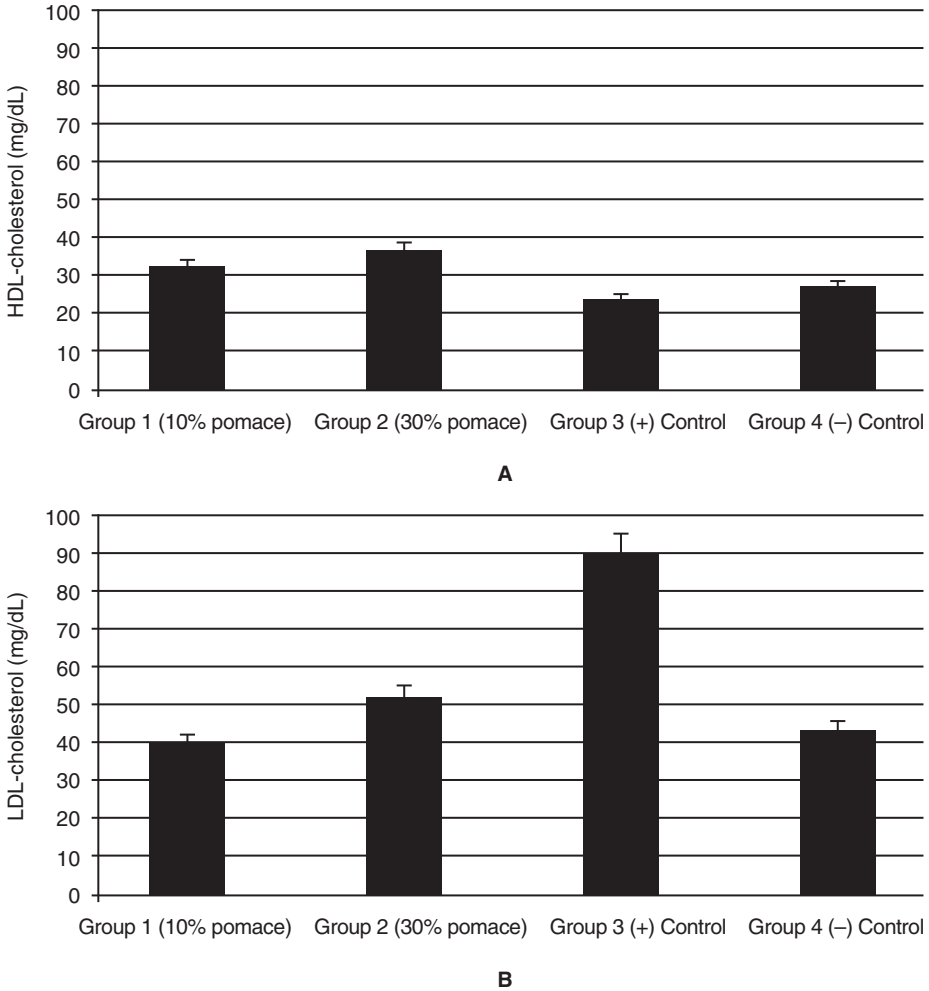

Figure 2
Impact of feeding goldenberry pomace on the levels of HDL-cholesterol (A) and LDL-cholesterol (B). Values given are the mean of three replicates and error bars show the variations of three determinations in terms of standard deviation

Increased plasma low density lipoprotein (LDL) concentration is associated with the susceptibility to developing atherosclerosis (Penumathsa *et al*., 2007). The oxidation of lipids and proteins in lipoproteins and cell membranes leads to the impairment of lipid transport and to cell injury which contributes to the development of various diseases. LDL carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries. The rise in LDL may cause a deposition of cholesterol in the arteries and aorta and hence is bad for health and a direct risk factor for CHD (Ramadan *et al*., 2008a). The accumulation of LDL within the arterial walls appears to play a crucial role in the initiation and progression of atherosclerotic plaque. After two months, data showed that LDL levels were significantly decreased (Figure 2B) in all treatments compared to the control groups. The highest decrease (*ca.* 54% compared to HCD control group) in LDL was detected in group 1 followed by group 2. These results suggest that goldenberry pomace may be preventative against atherosclerosis.

3.3. Liver functions

Figure 3A shows the effect of administering goldenberry pomace on the liver functions (GPT and GOT) of hypercholesterolemic rats. The transaminases GOT and GPT merely give a crude estimate of the degree of liver damage. Hypercholesterolemia (group 3) was characterized by a significant increase in serum GPT. The highest level of GPT was 14.8 u L^{-1} in the positive control group, while the levels were 11-11.3 u L^{-1} in groups fed the pomace. This increase in serum GPT activity indicates liver cell necrosis and hepatic injury. Treatment with pomace induced a decrease in the high activity of serum GPT and the levels were decreased compared to the positive control group after administration for 60 days. Also, hypercholesterolemia (group 3) was characterized by an increase in serum GOT. The increase of serum GOT activity was more specific for cardiac injury. The amounts of serum GOT were 9.25-10.1 u L^{-1} in the groups fed pomace. The highest amount of GOT was 13.6 u L^{-1} in the positive control group. Serum GOT and GPT were high as shown as Figure 3A and the data were in agreement with those of Daher *et al*. (2006).

Hypercholesterolemic state was accompanied by a high increase in total serum protein, albumin and globulin (Figure 3B). There was a remarkable decrease for the groups treated with pomace. The total protein value for group 3 (positive control) was 7.5 g dL^{-1}, while the values were lower for the groups treated with pomace (5.8 g dL^{-1}). Concerning serum albumin, the groups treated with pomace also recorded lower amounts (2.9-3.0 g dL^{-1}), while the positive control group recorded the highest amount (4.8 g dL^{-1}). Globulin levels also reached their highest levels for the positive control group, while the negative control group recoded the lowest level.

The hyperlipidemia was accompanied by an increase in kidney function parameters. Feeding with fruit pomace resulted in a reduction in uric acid and urea levels at the end of the experiment. The serum uric acid (Figure 3C) level was the highest for the positive control group (4.94 mg dL^{-1}). Blood urea is a major nitrogenous component of the urine. An increased urinary concentration of urea was observed in the states with pronounced catabolism of proteins and other nitrogenous components (starvation, burns, traumatism and atrophy of tissues, etc.). A decreased excretion of urea is observed in affected liver (urea-producing organ) and in impaired glomerular filtration of blood plasma. According to the obtained results presented in Figure 3C, the rat groups fed goldenberry pomace recorded lower urea content (33.2-37.5 mg dL^{-1}) after 60 days of feeding in comparison with the positive control group (51.4 mg dL^{-1}), while the negative group reached 40.6 mg dL^{-1}.

3.4. Liver and kidney histological examination

The gross appearance of the liver from rats fed with the normal diet, HCD and HCD plus goldenberry pomace was monitored. The liver of the control (negative) rats had a relatively dark-red color whereas the HCD-fed rats had an enlarged liver with a yellowish color. The weight of liver in the normal dietfed rats was 13.22 ± 0.50 g and in the HCDfed rats it was 20.39 ± 0.74 g, whereas in the HCD plus goldenberry pomace-fed rats it was 17.15 ± 0.36 g. In the rats fed with HCD plus goldenberry pomace, there was not as much enlargement of the liver (with a relatively yellowish color) as was observed in the HCD-fed rats. Histological examination of the livers of the control rats and HCD with pomace-fed rats revealed intact cell architecture (Figures 4A-D). In contrast, the liver of the HCD-fed rats illustrated poor cellularity with extensive lipid depositions and enlarged hepatocytes (Figure 4B). In goldenberry pomace-fed rats (Figures 4C and 4D), a lesser degree of lipid deposition and hepatocytes enlargement was observed.

Figures 5A-D showed histological examination of the kidney of control rats and goldenberry pomace-fed rats. Kidney tubules of the negative control rats (Figure 5A) showed mild dilatation in the renal tubules of both cortex and medulla and degenerative changes in the epithelium of some tubules. In the positive control group (Figure 5B) kidney tubules showed mild dilatation in some tubules and degenerative changes in the epithelium of other tubules. In the group fed 10% goldenberry pomace (Figure 5C) tubules showed dilatation in moderate numbers of the renal tubules in both cortex and medulla, vacuolation of the epithelium of some renal tubules and perivascular edema. While in the group fed 30% goldenberry pomace (Figure 5D) tubules showed moderate tubular dilatation, degenerative changes in the tubular epithelium and perivascular edema. All groups were compared with the negative control group in which the liver hepatic tissue and kidney tubules were apparently normal.

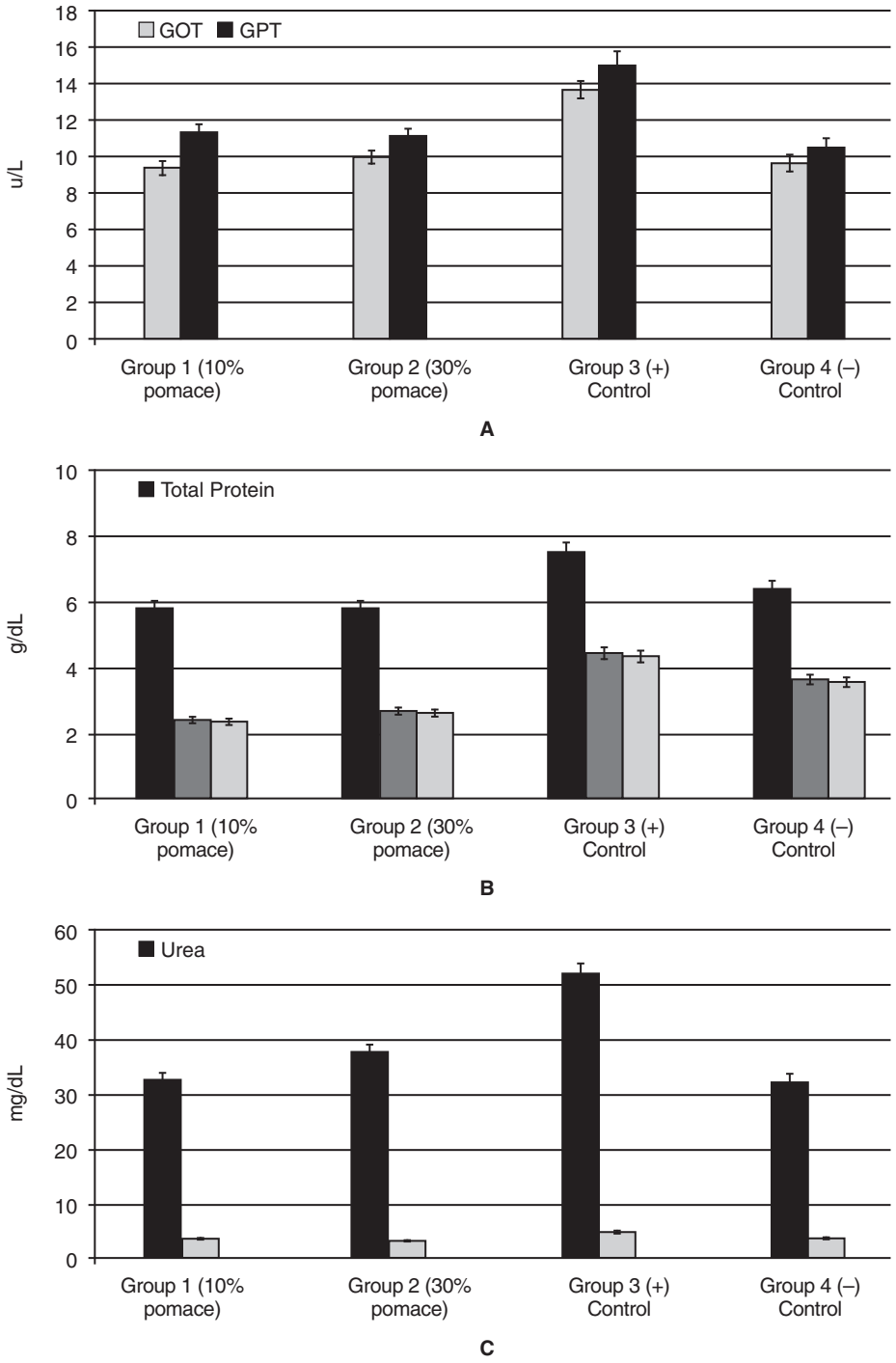

Figure 3
Impact of feeding pomace on the transaminases activity (A) serum protein fractions (B) and levels of urea and uric acid (C). Values given are the mean of three replicates and error bars show the variations of three determinations in terms of standard deviation

4. DISCUSSION

The classical concept of "adequate nutrition," a diet that provides nutrients in sufficient quantities to satisfy particular organic needs, is replaced by the concept of "optimal nutrition," which includes, besides nutrients, the potential of food to promote health and improve general well-being. This is where functional foods or medicinal foods, play their part (Nagai and Inoue, 2004).

Cholesterol can be good or bad depending on its concentration, circulation, accumulation and abnormal deposition within the body. On the one hand, cholesterol is essential as it has many functions in all animal life. First, cholesterol is an important compound in cell membranes as it regulates the membrane over a range of physiological temperatures. Second, cholesterol is a precursor for the synthesis of bile acids, which are emulsifiers of dietary fats and fat-soluble vitamins for digestion and absorption in

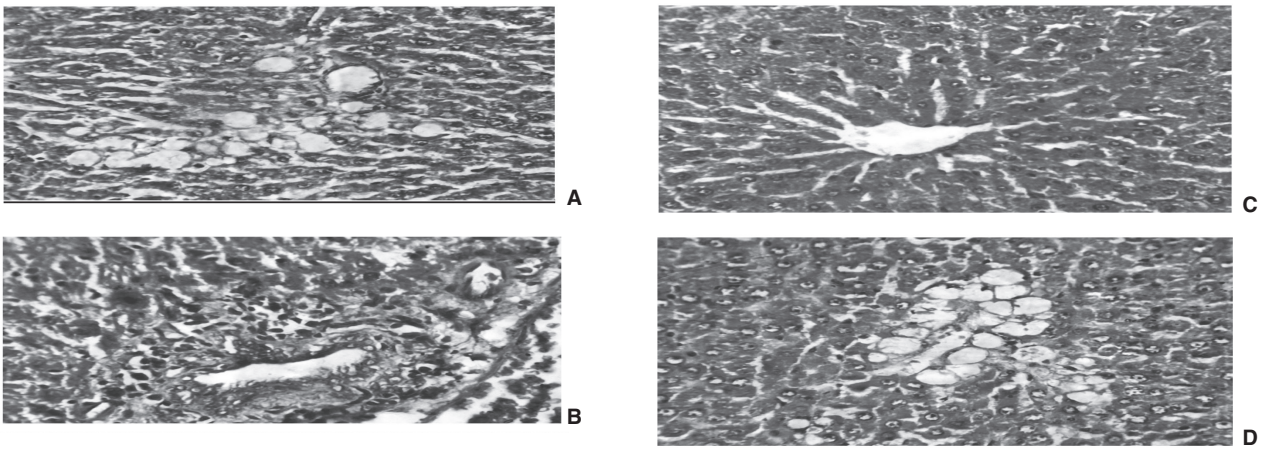

Figure 4
Hepatic cells from liver of negative control rats. B. Hepatic cells from liver of positive control rats. C. Hepatic cells from liver of rats treated with 10% goldenberry pomace. D. Hepatic cells from liver of rats treated with 30% goldenberry pomace

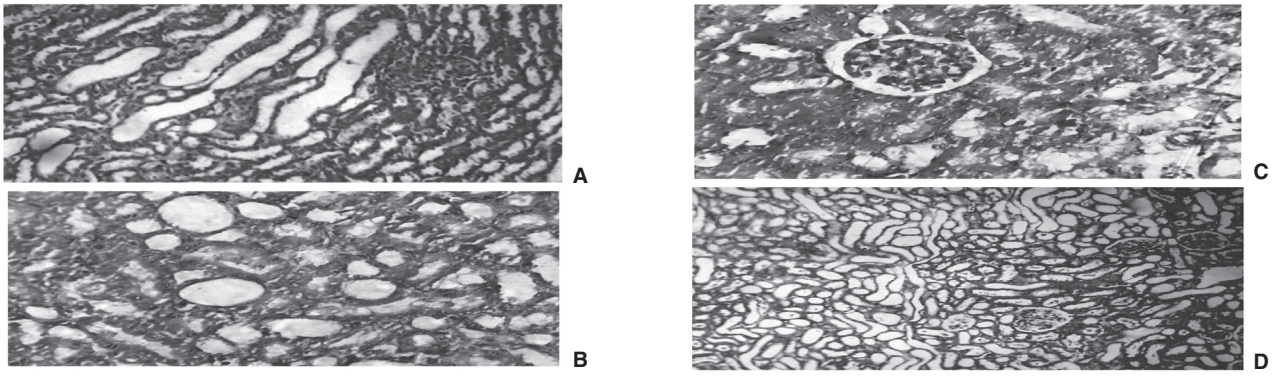

Figure 5
Kidney tubules of negative control rats. B. Kidney tubules of positive control rats. C. Kidney tubules of rats treated with 10% goldenberry pomace. D. Kidney tubules of rats treated with 30% goldenberry pomace

the small intestine. Third, it is a substrate for the synthesis of all steroidal hormones including sex hormones, glucocorticoids and mineralocorticoids. On the other hand, cholesterol is one of "culprits" which has been used an indication of general heart health. Epidemiological studies have demonstrated a strong association between plasma cholesterol concentration and CHD risk (Chen *et al*., 2011).

Hyperlipidaemia has been implicated in atherosclerosis, which is the leading cause of death among the world population. HCD increases serum LDL levels and oxidative stress which results in increases atherosclerotic plaque formation. People with CHD develop thickened or hardened arteries in the heart muscle. Efforts to develop effective and better hypolipidaemic drugs had led to the discovery of natural agents. Therefore, one of the aims of the present study was to evaluate the impact of goldenberry pomace as a hypolipidemic agent.

The hypocholesterolemic and atherosclerosprotective potentials of dietary consumption of goldenberry pomace were investigated by monitoring plasma lipid profiles in rats fed with either normal diet, HCD or HCD supplemented with fruit pomace. In the HCD-fed rats, an increased plasma TC and LDL-cholesterol with a decreased HDL-cholesterol was observed, and consumption of the goldenberry markedly suppressed the elevated TC and LDL levels plus increased HDL-cholesterol levels. Epidemiologic studies suggested that a high intake of fruit and vegetables is associated with a reduced risk of CHD (El-Anany and Ali, 2012).

The addition of fruit pomace to the diet resulted in the decrease of TC and LDL-cholesterol with an increase in HDL-cholesterol concentrations. This is probably due to the wealth of phytosterols. Numerous studies have noted that phytosterols induce a decrease in lipoprotein cholesterol levels in total plasma, but the mode of their action is not fully understood (Ramadan *et al*., 2011). It has been hypothesized that these compounds provoke a decrease in cholesterol solubility and their absorption across the intestinal barrier, inducing consequently low plasma cholesterol levels (Wasan *et al*., 2001). It has been demonstrated that these compounds prevent or delay the development of atherosclerotic lesions. The Fatty acid profile of fruit pomace which is rich in essential fatty acids as well

as PUFA may also play an important role for pomace health-promoting properties. Other bioactive compounds, such as tocopherols, are present in fruit pomace and could prevent the structural alteration of lipoproteins (Ramadan and Morsel, 2007; 2009). The beneficial effects could be also related to minor components, especially flavonoids, which are proposed to exert their action by inhibiting LDL oxidation and platelet aggregation, and caroteinoides, which are thought to act mainly as antioxidants (Kurowska *et al*., 2000).

Several possible mechanisms may be proposed to account for the improved hypercholesterolemia by goldenberry pomace. First, the fecal excretion pathway may be involved based on the observed decrease in lipid digestibility and increase in fecal cholesterol concentration. The *n*-3 fatty acids are reported to lower serum TAG levels (Craig, 1999). The liver plays an important role in the synthesis and net excretion of cholesterol either directly as free cholesterol in the bile or after conversion into bile acid (Choi *et al*., 2001). The rise in cholesterol in liver and plasma may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased cholesterol catabolism as evidenced by a reduction in bile acid production and turnover of bile acids. The metabolism of free and ester cholesterol are impaired in the liver, spleen and thymus tissue and the rate of turnover was specifically decreased in all tissues of hyperlipidemic rats (Feoli *et al*., 2003).

5. CONCLUSION

The consumption of cholesterol-enriched diet is regarded as an important factor in the development of CHD as it leads to the development of hyperlipidemia, atherosclerosis and abnormal lipid oxidation/metabolism. Thus, natural products with hypocholesterolemic and hypolipidemic properties may be useful in reducing the risk of CHD development. In this study, the effects of supplementation of goldenberry pomace on the lipoprotein-cholesterol profiles and vascular response of HCD-fed rats was evaluated. The results of *in vivo* experiment indicate that administration of fruit pomace has a profound influence on the metabolism of lipids in rats fed HCD. Fruit pomace positively affects the plasma lipid profile in rats fed HCD. The TC, LDL and TAG concentrations in the fruit pomace diet groups were lower than those in the positive control group. The positive influence on plasma lipids was high in the groups of rats fed pomace, which possesses high antioxidant potential. The results suggest that consumption of goldenberry pomace has hypocholesterolemic activities in rats fed HCD. In addition, goldenberry supplementation seems to protect the liver in response to oxidative stress as well as alleviate the magnitude of fatty liver development in response to HCD. It could be suggested that the use of goldenberry pomace by patients suffering from coronary atherosclerosis would prevent the development of this disease.

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