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RESEARCH ARTICLE

Protective Effects of Wheat Bran and Buckwheat Hull Extracts against Hypercholesterolemia in Male Rats

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Abstract

This study was designed to test wheat bran and buckwheat hull extracts as lipid-lowering and for their antioxidative activities in hypercholesterolemic rats. Buckwheat hull contain high amounts of total phenol, total flavonoid and tannins compared to wheat bran. Ferulic acid was the predominant phenolic acid found in wheat bran (695.32 µg/g dry weight). Rutin was found to be the major flavonoid presented in buckwheat hull (1255.7 µg/g dry weight). Buckwheat hull has the greatest DPPH and ABTS radical scavenging activity (90.36 and 93.90%, respectively) compared with wheat bran (68.68 and 73.24%, respectively). Rats fed on hypercholesterolemic diet and received wheat bran or buckwheat hull extracts at dose (1000 mg/kg body weight/day) showed significantly lowered in the plasma levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C) and glucose, while plasma high density lipoprotein (HDL-C) level showed slightly increase. The activity of transaminase enzymes (ALT and AST) significantly decreased in the groups administrated with wheat bran and buckwheat hull extracts compared with the hypercholesterolemic group (HC). Furthermore, the content of malondialdehyde (MDA) in plasma of treated groups showed a significantly decreased while total antioxidant capacity (TAC) was significant increased compared with HC group. In addition, wheat bran and buckwheat hull extracts improved plasma enzymatic antioxidants glutathione – S – transeferase (GST) and glutathione reductase (GR) in treated groups. In general, the results indicated that, both of wheat bran and buckwheat hull extracts showed significant effect in lipid lowering and antioxidative activities in hypercholesterolemic rats, but buckwheat hull extract was more effective than wheat bran extract. That might be due to its highly content of total phenol, total flavonoid and tannins (which showed a high antioxidant effect) with their abilities to lower plasma cholesterol level as well as to slow down the lipid peroxidation process and to enhance the antioxidant enzyme activity.

1. INTRODUCTION

Antioxidants are compounds that detoxify reactive oxygen species (ROS) and prevent their damage through multi mechanisms. Synthetic antioxidants have been in use as food additives for a long time, but reports on their involvement in chronic diseases have restricted their use in foods. Therefore, international attention has been focused on natural antioxidants mainly from plant sources (Dehghan et al., 2007 and Kai-Wei et al., 2009).

Natural antioxidants from plant extracts have attracted increasing interest due to consumer concern about the safety of the synthetic antioxidants in food. Extracts of fruits, vegetables, cereals and their by-products, such as the cereal hulls; all showed effective antioxidant activity in a model system (Sun and Ho, 2005).

Wheat bran, a by-product of flour milling is composed of the pericarp and the outermost tissues of the seed, including the aleurone layer. It constitutes almost 10% of the total weight of wheat milled for flour (Saunders et al., 1972).

Wheat bran is a good source of secondary metabolites as phenolic acids, flavonoids, lignans, phytosterols, tocopherols and tocotrienols and dietary fiber (Singh et al., 2012).

A large number of studies have shown that wheat whole grain and wheat bran extracts possess antioxidant properties against oxidation of biologically important molecules such as DNA, proteins and membrane lipids (Yu et al., 2005). Thus the consumption of whole wheat foods such as whole wheat bread or pasta besides other whole grain foods has been recommended for healthy diets (Wood, 2004).

Buckwheat (*Fagopyrum esculentum* Moench) is an alternative crop belonging to the Polygonaceae family and is usually grouped with cereals because of similarity in cultivation and utilization though it is pseudocereals not a cereal grain (Hung and Morita, 2008).

Buckwheat is one of the richest sources of polyphenols and flavonoids. These are concentrated mainly in the outer layers of buckwheat seed (Krkošková and Mrázová, 2005). These compounds protect the human organism against oxidative stress and prevent the development of chronic disease, e.g. atherosclerosis and neoplastic lesions (Hollman, 2001 and Kaliora and Dedoussis, 2007). They also indicate antibacterial, antiviral, anti-inflammatory and anti-allergenic action (Kim et al., 2003 and Kishore et al., 2010). Phenolic compounds are found mainly in the outer layers of buckwheat grains and their fraction comprises flavonoids and phenolic acids found in the free form and in ester and complex combinations (Hęś et al., 2012).

The present study was designed to compare the effect of wheat bran and buckwheat hull methanolic extracts in protecting experimental animals fed a hypercholesterolemia-induced diet of oxidative stress and hypercholesterolemia.

2. MATERIALS AND METHODS

2.1. Samples and chemicals

Wheat bran was purchased from the Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt. Buckwheat was purchased from health harvest market, 18 Anwar El Mofty St., Nasr City. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2\-azino-bis-(3-ethylbenzotiazoline-6-sulphonic acid (ABTS), sodium carbonate (Na_2CO_3), gallic acid, catechin and rutin were purchased from Sigma–Aldrich Chemical Co. (St. Louis, USA), Folin-Ciocalteu reagent was purchased from LOBA Chemie, India. All other chemicals used were of analytical reagent grade.

2.2. Preparation of wheat bran and buckwheat hull methanolic extracts

Ten grams of wheat bran and buckwheat hull powders were extracted with 100 ml methanol 80% at room temperature (25±1°C) overnight. The extracts were separated by filtering through filter paper Whatman, No. 1 and the procedure was repeated twice with 100 ml of solvent. The extracts (3×100ml) were combined and vacuum concentrated then freeze-dried. The two extract powders were stored at –20°C until used in the biological experiment.

2.3. Determination of total phenol

Total phenol was determined as described by Singleton and Rossi (1965). One gram sample was mixed with 10ml 80% methanol in a dark bottle and shaking for 2h. Then the mixture was filtrated. The color was developed by Folin-Ciocalteu reagent and sodium carbonate. 0.250 ml extract was mixed with 0.250 ml Folin-Ciocalteu reagent, 0.50 ml of 10% sodium carbonate (Na_2CO_3) and the volume was completed to 5ml with distilled water. After incubation in dark at room temperature for 30 min, the absorbance of the reaction mixture was measured at 725 nm against blank on a spectrophotometer (UV-Vis spectrophotometer, Labomed Inc., USA). Gallic acid was chosen as a standard to prepare the standard curve. Phenols were expressed as mg/100g sample on dry weight basis.

2.4. Determination of total flavonoid

Total flavonoid was determined according to the method of Zhishen et al. (1999). Sample (1g) was mixed with 10ml 80% methanol in a dark bottle and shaking for 2h. Total flavonoids extract (0.4ml) were added to 4ml H_2O. Then 0.3ml 5% $NaNO_2$ was added. After 5min 0.3ml 10% $AlCl_3$ was added. After 6min 2 ml of 1M NaOH were added and the total volume was made up to 10 ml with distilled water. The pink color was measured at 510nm against a blank reagent on a spectrophotometer (UV-Vis spectrophotometer, Labomed Inc., USA). Rutin served as

standard compound was used for preparing the calibration curve. Total flavonoid was calculated as mg/100g on dry weight basis.

2.5. Determination of tannins

Tannins were determined as described by Price et al. (1978). One gram sample was mixed with 10ml 1% methanol/HCl solution in dark bottle and shaking for 20min at room temperature. Then the mixture was filtrated. The tannins in the supernatant were estimated by using 1ml of supernatant and 5ml vanillin/HCl mixture (by mixing equal volumes of 2%vanillin in methanol and 8% methanol/HCl) in a test tube and kept for 20 min at room temperature. The formed color was determined at 500nm by using spectrophotometer (UV-Vis spectrophotometer, Labomed Inc., USA). Catechin was used to prepare the standard curve. Tannins were calculated as mg/100g on dry weight basis.

2.6. HPLC analysis of phenolic acid and flavonoid compounds in wheat bran and buckwheat hull

The phenolic acid and flavonoid compounds of the samples were extracted according to the method described by Hakkinen et al. (1998) and Mattila et al. (2000), respectively.

Ten grams sample were extracted using 10 ml of 80% methanol by homogenization for 2 min then centrifuged at 25,000 g for 10 min. The supernatant was decanted into a round-bottom flask. The pellet was re-suspended in 80% methanol (2x5ml) and centrifuged, and the supernatants were combined, filtrated through 0.20µm Millipore membrane filter and set up to a known volume. Three milliliters were collected in a vial for subsequent HPLC separation. HPLC instrument (Hewlett Packard, series 1050, country) equipped with column C18 hypersil BDS with particle size 5µm. Injection volume was 75 µl carried out with auto-sampling injector. The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate 1.0 ml/min. Elutes were monitored using UV detector set at 280 nm for phenolic acid and 330 nm for flavonoid. Chromatographic peaks were identified by comparing the retention times with the respective retention times of known standard reference material. Retention time and peak area were used to calculation of phenolic acid and flavonoid compounds concentration by the data analysis of Hewlett Packard software. Phenolic acid and flavonoid compounds were expressed as µg/g sample on dry weight basis.

2.7. Antioxidant activity

2.7.1. DPPH method

The antioxidant activity of plant methanol extracts was determined based on the radical scavenging ability in reacting with a stable DPPH free radical according to Brand-Williams et al. (1995). One gram sample was extracted with 10 ml 80% methanol for 2h as described above. Briefly, 2.4mg of DPPH in 100 ml methanol were prepared and 3.9 ml of this solution were added to 0.1 ml of methanolic extract. The mixture was shaken vigorously and allowed to stand in the dark for 30 min at room temperature. Then the absorbance was measured at 515 nm by using spectrophotometer (UV-Vis spectrophotometer, Labomed Inc., USA). Methanol was used as blank. The corresponding blank readings were taken and the capability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH^{\cdot} \text{ scavenging activity } (\%) = [(A0 - A1/ A0)] \times 100$$

Where: A0= The absorbance of the control reaction (containing all reagents except the test compounds).

A1= The absorbance in the presence of the tested extracts after 30 min.

2.7.2. ABTS radical cation scavenging assay

The ABTS assay was carried out according to Re et al. (1999). After preparing the ABTS radical (7mM, ABTS were dissolved in 10 ml deionized water), ammonium persulfate solution was prepared (2.45 mM in 10 ml). ABTS radical cation was produced by reacting 10 ml of ABTS stock solution with 10 ml ammonium persulfate then mixed, homogenized and kept in an amber flask for at least 16 h and protected from light. For the sample, an aliquot of 200 µl of the radical formed was pipetted and diluted in 10 ml 96° ethanol of analysis grade. The absorbance was measured at 734 nm by using spectrophotometer (UV-Vis spectrophotometer, Labomed Inc., USA). An aliquot of 980 µl of the diluted radical was pipetted and transferred to cuvette, 20 µl of the sample were added, homogenizing and agitating for a few seconds. The calculation of the radical inhibition percentage was made using the following equations:

$$ABTS^{\cdot} \text{ scavenging activity } (\%) = [(A0 - A1/ A0)] \times 100$$

Where: A0= The absorbance of the control reaction (containing all reagents except the test compounds).

A1= The absorbance in the presence of the tested extracts after 30 min.

2.8. Biological investigation

2.8.1. Experimental animals

Twenty adult male albino rats with an average weight of 140±5g were used in the present experiment. Rats were housed in polyethylene cages in Experimental Animal Unit, Food Technology Research Institute (FTRI), Giza, Egypt, under a controlled environment (25±2 °C, 50-60% relative humidity and 12-hour light-dark cycle). The rats were fed *ad libitum* according to AOAC (2000) with a basal diet consists of corn starch 60%, casein 20%, corn oil 10%, salt mixture 4%, vitamin mixture 1% and cellulose 5% and water for two weeks as adaptation period.

2.8.2. Experimental Design

After feeding on basal diet for two weeks, the rats were divided into 2 major groups. The first group (5 rats) was fed on the basal diet until the end of the experiment and was considered as a normal control group. The second group (15 rats) was fed on hypercholesterolemia-induced diet which prepared as basal diet preparation, except that the 10% corn oil portion was replaced with 10% sheep perianal fat and it was supplemented with 1% cholesterol and 0.25% cholic acid (Fukushima et al., 1997); after two weeks it divided into 3 groups. The first group, High-cholesterol control group (HC) fed on a hypercholesterolemia-induced diet until the end of the experiment. The other two groups fed on a hypercholesterolemia-induced diet until the end of the experiment and received methanolic extracts powder (1000 mg/kg b. wt/day) of wheat bran (WBE) and buckwheat hull (BWHE) after resolved in distillated water by stomach tube, respectively. During the experimental period (8 weeks), water and diets were available *ad libitum*.

At the end of the experiment, all the animals were scarified by cervical decapitation. Blood samples were collected in heparinized tube and centrifuged at 2500 rpm at 37°C for 15 min to separate the plasma.

2.9. Biochemical Analysis

2.9.1. Lipid Analysis

Plasma triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were determined according to Fossati and Prencipe (1982), Allain et al. (1974), Levy (1981) and Burstein (1970), respectively.

2.9.2. Determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities

AST and ALT activities were measured colorimetrically in plasma according to the method described by Reitman and Frankel (1957).

2.9.3. Determination of plasma Glucose

Plasma glucose level was determined according to Trinder (1969).

2.9.4. Determination of Lipid Peroxidation

Plasma lipid peroxidation was estimated by measuring the thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content, according to the method of Uchiyama and Mihara (1978). The MDA values were estimated using 1,1,3,3-tetraethoxy propane as the standard.

2.9.5. Determination of Enzymatic Antioxidant Activities

Plasma glutathione reductase (GR) activity was assayed by the method of Goldberg and Spooner (1983). The enzyme activity was quantified by measuring the disappearance of NADPH at 340 nm. Plasma glutathione-S-transferase (GST) activity was determined using the procedure of Habig et al. (1974) by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity.

2.9.6. Determination of total antioxidant capacity (TAC)

Plasma total antioxidant capacity (TAC) was assayed by method of Koracevic et al. (2001). The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2) the antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction which evolves the conversion of 3, 5, dichloro-2-hydroxy benzene sulphonate to a colored product.

2.10. Statistical Analysis

Data obtained from experimental animal studies were statistically analyzed according to Fisher (1970) by means of standard deviation (SD) and standard error (SE). Least significant difference (LSD) test was used to compare the significant differences between means of treatment according to Waller and Duncan (1969). The statistical package for social science SPSS (1999) program version was used for all analysis.

3. RESULTS AND DISCUSSION

3.1. The contents of total phenol, total flavonoid and tannins in wheat bran and buckwheat hull mg/100g sample

Data in Table 1 revealed that, in general buckwheat hull recorded the higher amounts of total phenol, total flavonoid and tannins than it found in wheat bran. Regarding total phenol content, in buckwheat hull was almost 5 times the amount found in wheat bran (1250.06 and 241.09 mg/100g sample in buckwheat hull and wheat bran, respectively). This results in the same trend with Vaher et al. (2010) who found that total phenolic content of fifteen wheat bran samples were ranged from (125.8 to 315.7 mg GAE/100g). Regarding to total phenol content in buckwheat hull Hęś et al. (2012) found that total phenolic content in buckwheat hull extracted by methanol was 16847 mg/100g dry matter. Danihelovà et al. (2013) found that total polyphenol in ten samples of buckwheat hull were in the range from 166.67 to 653.31 mg/100g of dry hull weight. That differentiation in total phenol content was explained by Danihelovà et al. (2013) who concluded that differences in polyphenol content between buckwheat varieties can be much higher because of varietal and environmental influences.

Total flavonoid was highly concentrated in buckwheat hull which contained over 16 times the amounts present in wheat bran (1168.09 and 69.34 mg/100g sample in buckwheat hull and wheat bran, respectively). These results are in accordance with Oomah and Mazza (1996) who published that the total flavonoid contents of four types of buckwheat hulls were ranged from 1212.8 to 1463.7mg /100g. Total flavonoids in four classes of wheat bran were ranged from 14.91 to 40.57 mg/100g (Feng and Mc Donald, 1989).

Tannin in buckwheat hull was 1.77 times of that found in wheat bran (85.05 and 47.93mg/100g sample in buckwheat hull and wheat bran, respectively). These results are comparable with that reported by Eggum et al. (1981) who reported that tannins content in two buckwheat varieties was significantly higher than its content in wheat.

3.2. Analysis of Phenolic acids and flavonoids in wheat bran and buckwheat hull on dry weight

Data in Table 2 showed that ferulic acid was predominating (80.45%) in wheat bran but protocatechuic acid was the highest phenolic acid (66.31%) found in buckwheat hull. The concentrations of syringic, caffeic, gallic, vanillic, *p*-hydroxybenzoic, *p*-coumaric, protocatechuic, ferulic and chlorogenic acids were 36.31, 11.20, 7.09, 47.15, 25.70, 28.52, 2.19, 695.32 and 10.79µg/g sample, respectively in wheat bran and 12.67, 43.75, 26.54, 27.47, 89.79, 31.65, 637.10, 42.09 and 49.69µg/g sample, respectively in buckwheat hull. These results are consistent with what was reported by Kim et al. (2006) ferulic, syringic and vanillic acids were the main phenolic acid extracted into the crude methanol extract of soft white wheat bran and ferulic acid was released in significantly higher amount. Wheat bran extracts content several phenolic acid including vanillic, *p*-coumaric and, largely, ferulic acid (Kähkönen et al., 1999). Also, Guo et al. (2012) reported that *p*-hydroxybenzoic, protocatechuic, caffeic, chlorogenic, gallic, ferulic, *p*-coumaric, syringic and vanillic acids were detected in buckwheat hull.

Regarding flavonoid derivatives (Table 3), flavonoids which identified in buckwheat hull namely rutin, quercitrin, quercetin, apigenin and kaempferol its values were 1255.7, 32.56, 4.367, 18.21 and 1.50µg/g, respectively. Rutin was the major components found in buckwheat hull. On the other hand, flavonoids content in wheat bran is very low. Apigenin was found to be the only flavonoid detected in wheat bran its value was 10.26µg/g. This result is consistent with (Zhang et al., 2010; Li and Zhang, 2001 and Dietrych-Szostak and Oleszek, 1999) they reported that, some phenolic compounds were isolated and identified from buckwheat including rutin, quercetin, quercitrin, kaempferol and apigenin. Also, Wijaya and Mares (2012) reported that Apigenin di-*C*-glycosides (ACGs) was present in the grain of bread wheat and other related cereals.

3.3. The antioxidant activities of wheat bran and buckwheat hull extracts using the DPPH and ABTS assay

DPPH is one of a few stable and commercially available organic nitrogen radicals and the scavenging radical of DPPH is based on the measurement of reducing ability of antioxidants toward DPPH (Huang et al., 2005 and Prior et al., 2005). The scavenging of the stable DPPH radical was widely used to evaluate antioxidant activity of phenolic compounds extracted from fruit, vegetable, cereal grain, wine, etc (Hung and Morita, 2008).

Data in Table 4 indicated that after 30 minutes, the DPPH scavenging of wheat bran extract was 68.68%, whereas the scavenging of buckwheat hull extract was 94.4% .These results indicated that in the DPPH assay, buckwheat hull extract had higher free radical scavenging activity than wheat bran extract.

Regarding ABTS radical cation decolorization assay which is applicable for both lipophilic and hydrophilic antioxidants, showed various radical scavenging activity between wheat bran and buckwheat hull. According to Table 4 buckwheat hull had the highest ABTS radical scavenging activity compared to wheat bran (93.90 and 73.24 %, respectively) and thus may be due to its higher content of total phenol and flavonoids compared to wheat bran.

These results are in the same trend with Kreft et al. (2006) who found that in comparison to antioxidative activity of the most frequently used cereals, buckwheat (*Fagopyrum esculentum* Moench) has been reported to possess higher antioxidant activity, mainly due to high phenolics content, namely rutin. Also Zielinski and Kozlowska (2000) have established the following hierarchy of antioxidant activity for 80% methanolic extracts originated from different whole grains: buckwheat > barley > oat > wheat = rye.

Gorinstein et al., 2007, Kishore et al., 2010 and Danihelovà et al., 2013 reported that positive relationship between the present polyphenolic compounds and their antioxidant activity has been found. Rutin appears to be the major antioxidant in buckwheat (Morishita et al., 2007).

In general, from these results it could be noticed that buckwheat hull with high content of polyphenols achieved the highest ability to scavenge free radicals. Also, we found a good correlation between polyphenol content and antioxidant activity in buckwheat hull and wheat bran. Buckwheat hull was higher in phenolics than wheat bran, for that its antioxidant activity as determined by binding free radical DPPH and ABTS exhibited better than wheat bran.

3.4. The effect of wheat bran and buckwheat hull extracts on the lipid profile of hypercholesterolemic rats

Results in Table 5 showed that fed on hypercholesterolemia-induced diet (HC) developed hypercholesterolemia mark by significant ($P < 0.05$) increase in plasma triglycerides (TG), total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C),and significant ($P < 0.05$) decrease in plasma high density lipoprotein cholesterol (HDL-C), compared to normal control rats (NC). Administrate with wheat bran and buckwheat hull extracts showed significant ($P < 0.05$) falls in TG, TC, LDL-C and significant increase in HDL-C compared to HC group.

It must be noticed that, the buckwheat hull extract is more effective than wheat bran extract for lowering lipids profile. The levels of TC, TG and LDL-C were decreased by 54.05, 52.73 and 57.75%, respectively in plasma of rats received buckwheat hull extract and also were decreased by 50.27, 50.23 and 51.73%, respectively in plasma of rats received wheat bran extract compared with hypercholesterolemic control. Slightly increase in HDL-C level was observed in groups administrated with buckwheat hull and wheat bran extracts compared with hypercholesterolemic control. Results showed that buckwheat hull extract was more effective against hypercholesterolemia than wheat bran extract. Our present findings are in line with Choi et al. (2007) who reported that oral administration of germinated buckwheat caused significant reductions in triglycerides, total cholesterol and LDL-cholesterol levels in serum and liver after eight weeks in mice fed with high fat diet. Rutin the main phenolic compounds of buckwheat extract show antioxidant and lipid peroxidation activities. It also has a lipid-lowering activity by decreasing the absorption of dietary cholesterol as well as lowering plasma and hepatic cholesterol (Zhang et al., 2012). Also, the effect of ferulic acid the predominant phenolic acid in wheat bran and found also in buckwheat hull reported by Sri Balasubashini et al. (2003) who found that supplementation with ferulic acid, which has been shown to have antioxidant properties, decreased the level of triglycerides and cholesterol in plasma. Ferulic acid is potent antioxidant and prevents LDL oxidation induced by copper ions; hence it facilitates the uptake and degradation of cholesterol by the liver.

3.5. The effect of wheat bran and buckwheat hull extracts on the transaminases activities and glucose of hypercholesterolemic rats

Data in Table 6 revealed that there were significant increase ($P < 0.05$) in the plasma AST and ALT activities of hypercholesterolemic rats (HC) as compared to normal control rats (NC). ALT and AST activities in the plasma significantly decreased in hypercholesterolemic rats received buckwheat hull and wheat bran extracts compared with hypercholesterolemic control (HC). The highest decrease in plasma ALT and AST activities were recorded in group received buckwheat hull extract. Free radicals are known to interact with cellular macromolecules, especially with unsaturated fatty acid, and initiate lipid peroxidation, thereby disrupting the structure and function of cell membrane. The measured serum transaminases (ALT and AST) levels are considered to be the most sensitive markers and, therefore usually employed for the diagnosis of hepatic injury. Since they are located in cytoplasm, and it is known that injury of the hepatocytes alters their transport function and membrane permeability leading to a leakage of

enzymes from the cells, rapidly after the cellular damage they are released into the blood circulation (Ramaiah, 2007). Rutin prevents changes in the activities of ALT and AST in the serum, liver and heart, indicating the protective effect of rutin against the hepatic and cardiac toxicity (Fernandes et al., 2010)

Plasma glucose level of hypercholesterolemic rats (HC) was significantly ($P<0.05$) increased compared to normal control group (NC). However, Significant decrease ($P < 0.05$) in plasma glucose by 15.19% of rats fed hypercholesterolemia-induced diet and received buckwheat hull extract (BWHE) compared to hypercholesterolemic control (HC). It was noticed that the effect of buckwheat hull extract was much better than the effect of wheat bran extract on reducing blood glucose level. This reduction in plasma glucose level can be explained by presence of ferulic acid and rutin in buckwheat hull and wheat bran extracts. These compounds found to be able to decrease high blood glucose to normal level (Sri Balasubashini et al., 2003 and Fernandes et al., 2010).

3.6. The effect of wheat bran and buckwheat hull extracts on the malondialdehyde (MDA) and total antioxidant capacity (TAC) levels of hypercholesterolemic rats

Several studies have demonstrated that high-fat diet increases oxidative stress in plasma and in a variety of tissues. Additionally high-fat diet was suggested to be a significant risk factor of cardiovascular disease (De Oliveira Otto et al., 2012).

Rats fed hypercholesterolemia-induced diet showed a significant ($P< 0.05$) increase in plasma malondialdehyde (MDA) level compared to the normal control rats (Table 7). MDA significantly increased ($P<0.05$) by 264.30% in hypercholesterolemic rats (HC) compared with normal control. Group of hypercholesterolemia-induced diet treated with buckwheat hull extract showed significant ($P<0.05$) decrease in MDA level compared to hypercholesterolemic rats. There was a slightly decrease but not significant in plasma level of MDA in hypercholesterolemic rats administrated with wheat bran extract compared with hypercholesterolemic control. Buckwheat hull extract was recorded the best results.

Lipid Peroxidation (LPO) refers to the reaction of oxidative deterioration of polyunsaturated lipids. Peroxidation involves the direct reaction of oxygen and lipid to form radical intermediates and to produce semi stable peroxides which, in turn, damage the enzymes, nucleic acids, membranes and proteins (Niki, 2009; Arukwe and Mortensen, 2011). MDA is the most abundant product of LPO, which is a common consequence of oxidative stress (Devaraj et al., 2008). Yan et al. (2012) showed that tartary buckwheat extracts supplementation groups significantly decreased the MDA levels. These results indicated that tartary buckwheat extracts could reduce lipid peroxidation and protected liver tissue from ROS-mediated oxidative damage. Also, Zhang et al. (2012) reported that MDA contents in serum and liver were decreased in all the treated groups administrated with total flavonoids from tartary buckwheat (FTB) extracts compared to the normal control group. The results indicated that oral administration with FTB could significantly reduce the lipid peroxidation and enhance the activity of antioxidant enzymes in mice. You et al. (2009) found that administration of ferulic acid efficiently activate the hepatic antioxidative defense system. The mice that received ferulic acid showed significant decrease in the MDA content.

Total antioxidant capacity (TAC) was measured in plasma of normal and hypercholesterolemic rats (Table 7) and the results showed a significant decrease by 42.94% in hypercholesterolemic control compared with normal control. The effect of buckwheat hull extract was much better than the effect of wheat bran extract on elevated TAC levels in treated groups (1.36 and 1.28mM/l, respectively).

3.7. The effect of wheat bran and buckwheat hull extracts on glutathione reductase (GR) and glutathione-S-transferase (GST) activity of hypercholesterolemic rats

Significant decrease in the activity of plasma glutathione reductase and glutathione-S-transferase (GST) (Table 8) was observed in the hypercholesterolemic control group (HC) compared to the normal control group (NC).

Plasma glutathione reductase activity (GR) was slightly increases by 13.05% and 2.32% in hypercholesterolemic rats received buckwheat hull and wheat bran extracts, respectively compared to hypercholesterolemic control (HC).

Also, slightly increase was observed in plasma glutathione-S-transferase activity (GST) in groups administrated with buckwheat hull and wheat bran extracts compared with hypercholesterolemic control.

Our results in the same trend with You et al. (2009) who reported that the mice that received ferulic acid, one of the most ubiquitous phenolic acids, found in the bran of grasses such as wheat, showed significant increases in the activity of hepatic antioxidant enzymes such as glutathione-S-transferase. Also, Yan et al. (2012) found that the high dose of tartary buckwheat extract supplementation groups significantly increased the GR activities.

Generally, buckwheat hull extract showed a significant effect in lowering lipid profile and increase the antioxidative activities on hypercholesterolemic rats compared with wheat bran extract that's may be due to its high content of total phenol, total flavonoid and tannins which showed a highly effects of antioxidant activity.

Our present findings are in line with (Wang et al., 2009; Fabjan et al., 2003 and Guo et al., 2011) whom stated that tartary buckwheat extracts can reduce total triglycerides and total cholesterol in the serum and liver, blood sugar and blood pressure, and raise serum antioxidant activity. Plenty of phenolic compounds contributing to the above beneficial effects in tartary buckwheat have been found, such as rutin, quercitrin, catechins, *p*-hydroxybenzoic, protocatechuic, gallic vanillic, *p*-coumaric, syringic, caffeic and ferulic acids.

Finally the present results clearly illustrate the possibility of using both of buckwheat hull and wheat bran extracts as hypocholesterolemic and antioxidative agents, but buckwheat hull was more effective than wheat bran as hypocholesterolemic agent, although further studies using higher concentrations of the hull may be needed to normalize the rest of the biochemical parameters. It is our opinion, however, that serious experiments must be carried out on human patients.

Table 1
Total phenol, total flavonoid and tannins (mg/100 g dwt.) in wheat bran and buckwheat hull

Samples	Total phenol (mg gallic acid equivalents /100g)	Total flavonoid (mg rutin equivalents/100g)	Tannins (mg catechin equivalents/100g)
Wheat bran	241.09^{b} ±0.81	69.34^{b}±4.45	47.93^{b} ±0.966
Buckwheat hull	1250.06^{a} ±1.61	1168.09^{a} ±13.50	85.05^{a} ±3.24
LSD 0.05	4.989	39.455	5.4199

Table 2
Phenolic acids composition (µg/g sample) in wheat bran and buckwheat hull on dry weight

Compounds	Wheat bran (µg/g dwt)	Buckwheat hull (µg/g dwt)
Syringic acid	36.31	12.67
Caffeic acid	11.20	43.75
Gallic acid	7.09	26.54
Vanillic acid	47.15	27.47
p-Hydroxybenzoic acid	25.70	89.79
p-Coumaric acid	28.52	31.65
Protocatechuic acid	2.19	637.10
Ferulic acid	644.32	42.09
Chlorogenic acid	10.79	49.69

Table 3
Flavonoids composition (µg/g sample) in wheat bran and buckwheat hull on dry weight

Compounds	Wheat bran (µg/g dwt)	Buckwheat hull (µg/g dwt)
Apgenin	10.26	18.21
Rutin	—	1255.7
Quercitrin	—	32.56
Quercetin	—	4.367
kaempferol	—	1.50

Table 4
Antioxidant activities (%) of wheat bran and buckwheat hull using the DPPH and ABTS assay

Sample	DPPH (%)	ABTS (%)
Wheat bran	68.68^{b} ±0.60	73.24^{b} ±0.81
Buckwheat hull	90.36^{a} ±0.70	93.90^{a} ±0.94
LSD 0.05	2.557	3.446

Table 5
Plasma total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol (mg/dl) in rats fed hypercholesterolemia-induced diet and received wheat bran and buckwheat hull extracts

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
NC	91.06^{c} ±4.54	91.21^{c} ±3.22	55.66^{a} ±1.29	40.23^{c} ±8.39
HC	402.59^{a} ±39.80	328.97^{a} ±16.73	42.90^{b} ±1.71	293.89^{a} ±39.05
WBE	200.02^{b} ±8.14	163.74^{b} ±10.05	45.40^{b} ±3.27	141.85^{b} ±9.43
BWHE	185.01^{b} ±9.99	155.51^{b} ±5.20	49.90^{ab} ±2.13	124.17^{b} ±9.65
LSD	63.080	30.650	6.674	63.192

Values are expressed as means ± SE (n = 5).
Values with different superscript letters within the same column are significantly different (P < 0.05)
NC: Normal Control
HC: Hypercholesterolemic control.
WBE: Wheat bran extract.
BWHE: Buckwheat hull extract.

Table 6
Plasma AST, ALT activities (U/l) and glucose (mg/dl) in rats fed hypercholesterolemia-induced diet and received wheat bran and buckwheat hull extracts

Groups	AST (U/l)	ALT (U/l)	Glucose (mg/dl)
NC	21.82^{c} ±2.12	20.72^{c} ±1.06	84.54^{c} ±3.01
HC	87.22^{a} ±0.75	39.09^{a} ±0.57	115.16^{a} ±6.94
WBE	54.10^{b} ±2.99	33.68^{b} ±1.30	103.32^{ab} ±2.06
BWHE	50.63^{b} ±2.90	31.45^{b} ±1.18	97.66^{bc} ±4.15
LSD 0.05	7.099	3.198	13.299

Values are expressed as means ± SE (n = 5).
Values with different super script letters within the same column are significantly different (P< 0.05).
NC: Normal Control
HC: Hypercholesterolemic control.
WBE: Wheat bran extract.
BWHE: Buckwheat hull extract.

Table 7
Plasma MDA (nmol/L and TAC (mM/l) in rats fed hypercholesterolemia-induced diet and received wheat bran and buckwheat hull extracts

Groups	MDA (nmol/L)	TAC (mM/l)
NC	22.61^{c}±3.31	1.70^{a}±0.22
HC	82.37^{a}±2.01	0.97^{b}±0.31
WBE	73.92^{ab}±3.01	1.28^{a}±0.21
BWHE	68.37^{b}±3.69	1.36^{a}±0.21
LSD 0.05	9.205	0.722

Values are expressed as means ± SE (n = 5).
Values with different super script letters within the same column are significantly different (P< 0.05).
NC: Normal Control
HC: Hypercholesterolemic control.
WBE: Wheat bran extract.
BWHE: Buckwheat hull extract.

Table 8
Plasma GR and GST (U/L) activities in rats fed hypercholesterolemia-induced diet and received wheat bran and buckwheat hull extracts

Groups	GR (U/L)	GST (U/L)
NC	41.50^{a}±2.25	45.37^{a}±1.90
HC	29.80^{b}±0.49	36.16^{b}±1.41
WBE	30.49^{b}±1.81	36.819^{b}±1.03
BWHE	33.69^{b}±3.09	37.76^{b}±3.05
LSD 0.05	6.382	5.99

Values are expressed as means ± SE (n = 5).
Values with different super script letters within the same column are significantly different (P< 0.05).
NC: Normal Control
HC: Hypercholesterolemic control.
WBE: Wheat bran extract.
BWHE: Buckwheat hull extract.

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