Australian Journal of Basic and Applied Sciences, 5(10): 30-37, 2011
ISSN 1991-8178

Hypolipidemic and Antiatherogenic Effects of Dietary Chitosan and Wheatbran in High Fat- High Cholesterol Fed Rats.

Lamiaa, A.A. Barakat

Department of Biochemistry and Nutrition, Women College, Ain Shams University, Cairo, Egypt.

Abstract: Background: Dietary modification contributes significantly in reducing cardiovascular disease (CVD) risk factors including lowering cholesterol and atherosclerosis. **Aims:** The purpose of this study was to examine the efficiency of using either chitosan or wheat bran or a mixture of equal amounts of chitosan and wheat bran on the prevention of hyperlipidemia, hypercholesterolemia – induced in rats fed high fat, high cholesterol diets. **Materials and Methods:** 80 male albino rats were divided into eight groups: (1) normal control; animals in groups 2, 3 and 4 were fed on basal diet supplemented with chitosan or wheat bran or chitosan and wheat bran at equal amounts (5%), respectively, (5) control hypercholesterolemic-hyperlipidemic rats, fed the balanced diet supplemented with cholesterol and fats at a dose level of 1 g and 20g/100 g diet respectively; then the other three groups of animals fed the same previous hypercholesterolemic diet supplemented with chitosan (6) or wheat bran(7) or chitosan and wheat bran at the same level (5%)(8). **Results** The present study showed that 1% cholesterol and 20% fat administration caused a significant increase in total cholesterol, total lipids, and triacylglycerols in both serum and liver. Serum phospholipids, LDL-C, and atherogenic index (AI) also significantly increased compared to normal control group. Cholesterol-enriched diet significantly increased fecal total total lipids, total cholesterol and triacylglycerol levels as well as significantly increased serum malondialdehyde and significantly decreased blood glutathione, glutathione peroxidase and liver nitric oxide compared to healthy control. Consumption of chitosan, wheat bran or a combination mixture of them by healthy and hyperlipidemic hypercholesterolemic rats resulted in a significantly decrement in lipid parameters in serum and liver and significant improvement antioxidant enzymes as compared with their corresponding control rats. **Conclusion**: These results suggest that both chitosan and wheat bran had anti-atherogenic hypolipidemic and reduced oxidative stress via inhibition of reactive oxygen species and lipid peroxidation as well as increment of antioxidant enzymes.

Key words: Hypercholesterolemia, Chitosan, Wheat bran, Lipid peroxidation, Fecal fats and oxidative stress.

INTRODUCTION

Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia, is a major risk factor for the development of cardiovascular diseases. The search for new drugs able to reduce and/ or to regulate serum cholesterol and triacylglycerol levels has gained importance over the years, resulting in numerous reports on significant activities of natural agents (Jahromi *et al*., 1993 and Makni *et al*., 2008). Although plant extracts constitute potential candidates, they often contain complex mixture of many different compounds with distinct polarity, antioxidant and pro-oxidant properties (Kahkonen *et al*., 2001; Parejo *et al*., 2002 and Makni *et al*., 2008). The beneficial effects of dietary fiber have attracted strong attention. These benefits are not only recognized as being a reduction in the energy density of a diet and an increase in the stool weight or in the frequency of defecation, but also as a preventive measure against disorders prevalence in the lower intestinal tract, e.g., diverticulitis or colon cancer (Deuchi *et al*., 1994). Fibers are reported to decrease plasma LDL-C levels by interrupting the cholesterol and bile acid absorption and increasing LDL receptor activity. In fact dietary fiber are known to interfere with cholesterol absorption and enterohepatic bile circulation and resulted in depletion of hepatic cholesterol pools (Romero *et al*., 2002; Lecumberri *et al*., 2007). In addition, diets rich in fibers are known to reduce triacylglycerol levels by inhibition of hepatic lipogenesis (Venkatesan *et al*., 2003). Chitosan, although not derived from plants, is similar to dietary fiber in being a polysaccharide that is indigestible by mammalian digestive enzymes. Chitosan is a derivative of chitin, natural polymer of glucosamine and N-acetylglucosamine derived from the shells of crustaceans such as crab, lobster and shrimp. Because of its chemical structure, β-1, 4-linked polymer of D-glucosamine, chitosan does not get broken down or digested by human gastrointestinal enzymes. It is the most abundant natural polymer after cellulose (Muzzarelli, 2000; Rockway and Menard, 2000; Tapola *et al*., 2008).

Chitosan has a positively charged tertiary amino group (β-NH^{3+}) to which negatively charged molecules like fatty and bile acids can strongly attach. Chitosan also binds neutral lipids like cholesterol and triglycerides

Corresponding Author: Lamiaa, A.A. Barakat, Department of Biochemistry and Nutrition, Women College, Ain Shams University, Cairo, Egypt.

through hydrophobic bonds. In humans, dietary chitosan has been reported to reduce serum total cholesterol levels from 5.8% to 42.6% and low-density lipoprotein (LDL) levels from 15.1% to 35.1% (Ylitalo *et al*., 2002 and Tapola *et al*., 2008).

The objective of this study was to examine the effect of chitosan, wheat bran and a combination of the two, on cholesterol absorption and fat excretion to understand how these materials lower cholesterol. The effect of these materials on fat excretion was also determined as a possible explanation for the observation in several studies that chitosan supplements accelerated weight loss in subjects consuming hypocaloric diets (Sciutto and Colombo 1995, Veneroni *et al*., 1996 and Gallaher *et al*., 2000).

MATERIALS AND METHODS

Animals:

This study was carried out on healthy adult male albino rats (Sprague-Dawley) strain weighing 120 ± 5 g, supplied from the breeding unit of the Egyptian organization for biological products and vaccines (Helwan, Egypt), rats were housed of ten per cage under controlled environment conditions cycle. Rats were fed experimental diet (Revees *et al*, 1993) and were given tap water ad libitum throughout the experimental work. The experimental animals divided into two sections the healthy rats and the hyperlipidemic, hypercholesterolemic one(HF-HC).

Experimental Design:

Animals randomly enrolled into eight groups:

Groups (1) normal control; Animals in groups 2, 3 and 4 were fed on basal diet supplemented with chitosan or wheat bran or a combination of chitosan and wheat bran at equal amounts (5%), respectively, (5) control hypercholesterolemic-hyperlipidemic rats, fed the balanced diet supplemented with cholesterol and fats at a dose level of 1 g and 20g/100 g diet respectively; then the other three groups of animals fed the same previous hypercholesterolemic hyperlipidemic diet supplemented with chitosan (6) or wheat bran(7) or chitosan and wheat bran at the same level (5%)(8). At the end of experimental period (6 weeks), and after over night fasting (12 hours), rats were scarified under ether anesthesia and blood samples were collected from hepatic portal vein in two centrifuge tubes. One tube contained the serum which was separated by allowing blood samples left for 15 minutes at temperature of 25°C then centrifuged at 4000 r.p.m for 20 minutes by EBA8 centrifuge (Made In China), then kept in plastic vials at −20°C until analysis of total cholesterol by the methods of Allian *et al*., (1974), HDL-cholesterol by Burstein *et al*., (1970), triacylglycerol by Fossati and Prencipe (1982), total lipids by Zollner and Kirsch, (1962),phospholipids by Takayama *et al*.(1977) **.** Calculation of LDL-C fraction and atherogenic index (AI) and HTR ratio involves an equation developed by Friedewald *et al*., (1972)**.** Lipid peroxidation was estimated in serum by measuring the malondialdehyde (MDA) production formed in the thiobarbituric acid reaction (Mihara and Uchiyama, 1978).The second tube contained heparinized blood used for determination glutathione concentration by the method of Beutler *et al*., (1982). Glutathione peroxidase (GPX) activity was assayed in erythrocytes by a coupled test system, in which glutathione reductase is employed for regeneration of GSH and butylhydroperoxide used as the acceptor substrate. The decrease in NADPH concentration was registered photometrically at 340 nm (Günzler *et al*., 1974). Nitric oxide was determined in the liver according to Montgomery and Dymock (1961).

A-3 day fecal collection was made in the last week, dried and kept frozen until lipid extraction.

Tissue and fecal lipids were extracted with chloroform/ methanol mixture (2v/1v) according to the method of Folch *et al*., (1957). The dried total lipids residues were dissolved in 1 ml absolute ethanol for total lipids, total cholesterol and triacylglycerol assays. Hepatic and fecal total lipids, total cholesterol and triacylglycerol contents were analyzed with the same enzymatic kits used in serum analysis.

Statistical Analysis:

The results were expressed as means ± S.D. and analyzed for statistical Significance by two-way ANOVA followed by tukey's post-hoctest for multiple comparisons, using SPSS program for windows version 15.0 (Spss Inc, Chicago, USA). Values were considered statistically significant at $p < 0.05$.

Results:

Effect of Chitosan, Wheat Bran and the Combination Mixture of The Two on Body Weight and Relative Organs Weight:

As shown in Table 1 induced hypercholesterolemia and hyperlipidemia(HF-HC diet) caused a significant increase in body weight gain and a significant increase in the relative organs weight as compared with healthy control G1. Administration of chitosan, wheat bran and the combination mixture of equal amounts of them to either normal or hyperlipidemic, hypercholesterolemic rats caused a significant decrease in body weight gain and in the relative organs weight as compared with their corresponding control rats.

Table 1: Effect of supplementing chitosan, wheat bran or the combination of two on body weight gain and relative organs weight in hypercholesterolemic rats.

Groups	Body weight gain (g)	Relative weight of liver (mg/g tissue)	Relative weight of Heart (mg/g tissue)	Relative weight of Kidney(mg/g tissue)
G1 (control)	119.13±3.98^{bc}	4.45±0.24^{abc}	0.38±0.04 ^{bc}	0.47±0.03 ^{abc}
G2 (Healthy+chitosan	110.63±5.13^{d}	3.91±0.2^{d}	0.35±0.01 ^{c}	0.42±0.02 ^{c}
G3(Healthy+wheat bran	117.5±2.67^{c}	4.36±0.13^{bc}	0.38±0.02 ^{bc}	0.44±0.04 ^{bc}
G4(Healthy+ chitosan+wheat bran	113.75±3.2^{cd}	4.27±0.14^{c}	0.38±0.08 ^{bc}	0.44±0.03 ^{bc}
HF-HC control (G 5)	166.5±7.48 ^{a}	4.7±0.18 ^{a}	0.45±0.06 ^{a}	0.52±0.05 ^{a}
HF-HC+chitosan(G 6)	118.38±10.23 ^{c}	4.43±.24^{bc}	0.35±0.03 ^{c}	0.46±0.07 ^{bc}
HF-HC+wheat bran (G 7)	127.75±6.2 ^{b}	4.53±0.37^{ab}	0.4±0.04 ^{b}	0.49±0.08 ^{ab}
HF- HC+ (G 8) chitosan+Wheatbran	124.88±3.91^{b}	4.52±.39^{abc}	0.39±0.04 ^{bc}	0.47±0.06 ^{abc}
L.S.D	5.86	0.26	0.050	0.054

Effect of Chitosan, Wheat Bran and the Combination Mixture of the Two On Serum, Liver and Fecal Lipid Contents:

As shown in tables (2) (3) and (4), feeding rats with 20 % fat and 1% cholesterol-enriched diet for 6-weeks resulted in a significant elevation of serum and liver total cholesterol (111.65%; 43.32%), total lipids (16.89% and 39.18%) and triacylglycerols (4.12%; 43.56%). Serum phospholipids, LDL/HDL ratio and AI were also significantly increased in HF-HC control group .Fecal lipids were also significantly increased and influenced by the amount of fat in the diet. Administration of chitosan, wheat bran or the combination mixture of equal amounts of them to either normal or hyperlipidemic, hypercholesterolemic rats caused a significant decrease in the levels of serum and liver total lipids, total cholesterol and triacylglycerols, however, fecal fat excretion was significantly increased by these fiber supplementation. The HTR% increased while AI was significantly decreased in HF-HC rats fed fibers as compared to those of hypercholesterolemic control group. It was noticed that the lowering effect of chitosan on serum and hepatic total lipids was more observable than that of wheat bran (chitosan>combination mixture>wheat bran).

Effect of Chitosan,WwheatBbran and the Combination Mixture of the two on Serum MDA, Blood GSH and GPx and Liver NO:

Feeding rats with 20% fat and 1% cholesterol caused a significant increase in serum MDA by 144.69% and a significant depletion in the values of whole blood GSH, GPx and liver NO by about 21.67%, 33.9% and 44.9% respectively as compared with healthy control group. Serum MDA was significantly decreased both in healthy and HF-HC rats by fiber supplementation. Moreover, chitosan, wheat bran or the combination of two were significantly increased blood GSH, GPx and liver NO either in healthy or HF-HC rats as compared with their corresponding control.

Table 2: Effect of supplementing chitosan, wheat bran or the combination of two on lipid parameters and atherogenic index in healthy and HF-HC rats.

Groups	Total Cholesterol (mg/dL)	Total lipids (mg/dL)	Triacylglycerol (mg/dL)	Phospholipids (mg/dL)	Atherogenic index
G1 (control)	99.45±1.49 ^{e}	338.7±0.82^{cd}	68.79±0.79^{e}	138.7±0.82 ^{d}	1.57±0.05 ^{e}
G2 (Healthy+chitosan	89.98±0.47^{g}	260.56±16.94 ^{f}	61.03±0.63^{h}	113.82±3.1 ^{e}	1.22±0.09^{g}
G3(Healthy+wheat bran	93.47±0.66 ^{f}	295.76±7.07^{e}	64.34±0.82^{f}	118.26±0.94 ^{e}	1.44±0.07^{ef}
G4(Healthy+ chitosan+wheat bran	91.59±1.05 ^{fg}	270.01±14.98^{f}	62.87±0.53^{g}	115.18±2.83 ^{e}	1.35±0.03 ^{fg}
HF-HC control (G 5)	228.3±4.07 ^{a}	525.72±3.2 ^{a}	103.9±1.42^{a}	225.72±3.21 ^{a}	8.74±0.39^{a}
HF- HC+chitosan (G 6)	146.07±2.81 ^{d}	333.63±8.6 ^{d}	74.19±2.07^{d}	168.51±3.3 ^{c}	2.56±0.12 ^{d}
HF-HC+wheat bran(G 7)	170.68±2.75 ^{b}	383.76±7.57 ^{b}	85.99±2.08^{b}	196.01±5.07 ^{b}	4.67±0.22 ^{b}
HF- HC+ (G 8) chitosan+Wheatbran	149.68±2.24 ^{c}	344.05±7.35 ^{c}	76.76±1.94^{c}	168.05±10.13 ^{c}	2.79±0.13 ^{c}
L.S.D ($p<0.05$)	2.67	9.75	1.43	4.6	0.18

Table 3: Effect of supplementing chitosan, wheat bran or the combination of two on VLDL-C, HDL-C, LDL-C, and HTR ratio in healthy and HF-HC rats.

Groups	VLDL-C (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	LDL-C / HDL-C ratio	HTR ratio
G1 (control)	13.67±0.16 ^{d}	38.7±0.82 ^{cd}	46.99±1.4 ^{d}	1.22±0.05 ^{e}	38.92±0.83^{d}
G2 (Healthy+chitosan	12.21±0.13 ^{g}	40.56±1.82^{ab}	37.21±1.66 ^{f}	0.92±0.08 ^{g}	45.08±1.94^{a}
G3(Healthy+wheat bran	12.87±0.16 ^{e}	38.26±0.94 ^{d}	42.39±1.18 ^{e}	1.11±0.06 ^{e}f	40.94±1.11^{c}
G4(Healthy+ chitosan+wheat bran	12.57±0.11 ^{f}	39.01±0.74 ^{cd}	40.01±o.67 ^{e}	1.03±0.02^{fg}	42.59±0.55 ^{b}
HF-HC control (G 5)	20.78±0.28 ^{a}	23.47±0.93 ^{f}	184.05±4.19 ^{a}	7.85±0.36 ^{a}	10.28±0.43^{h}
HF- HC+chitosan (G 6)	14.84±0.41 ^{d}	41.13±1.83 ^{a}	90.11±2.25 ^{d}	2.19±0.1 ^{d}	28.15±0.97 ^{e}
HF-HC+wheat bran(G 7)	17.2±0.42 ^{b}	30.13±0.91 ^{e}	123.34±3 ^{b}	4.1±0.2 ^{b}	17.66±0.72 ^{g}
HF- HC+ (G 8) chitosan+Wheatbran	15.35±0.39 ^{c}	39.55±0.77 ^{bc}	94.77±3.25 ^{c}	2.4±0.13 ^{c}	26.44±0.89 ^{f}
L.S.D ($p<0.05$)	0.29	1.18	2.47	0.16	1.03

Table 4: Effect of supplementing seed mixtures on hepatic total cholesterol, total lipids and triacylglycerols in healthy and HF-HC rats.

Groups	Total Cholesterol Mg/g tissue	Total lipids Mg/g tissue	Triacylglycerol Mg/g tissue
G1 (control)	3.37±0.07 ^{e}	38.7±0.82 ^{d}	5.33±0.16 ^{de}
G2 (Healthy+chitosan	2.93±0.14 ^{g}	35.2±1.47 ^{e}	4.68±0.21 ^{g}
G3(Healthy+wheat bran	3.13±0.04 ^{f}	36.84±1.58 ^{de}	5.13±0.04 ^{ef}
G4(Healthy+ chitosan+wheat bran	3.03±0.03 ^{fg}	36.3±0.89 ^{e}	5.03±0.06 ^{f}
HF-HC control (G 5)	7.33±0.26 ^{a}	77.85 ±3.71 ^{a}	8.3±0.25 ^{a}
HF- HC+chitosan (G 6)	4.43±0.35 ^{d}	61.63±1.65 ^{c}	5.52±0.18 ^{cd}
HF-HC+wheat bran(G 7)	5.82±0.2 ^{b}	68.01±1.68 ^{b}	6.57±0.48 ^{b}
HF- HC+ (G 8) chitosan+Wheatbran	4.63±0.17 ^{c}	63.3±1.97 ^{c}	5.63±0.14 ^{c}
L.S.D ($p<0.05$)	0.19	1.92	0.23

Table 5: Effect of supplementing chitosan, wheat bran or the combination of two on fecal total cholesterol, total lipids and triacylglycerols in healthy and HF-HC rats.

Groups	Total Cholesterol Mg/g	Total lipids Mg/g	Triacylglycerol Mg/g
G1(control)	13.06±0.2 ^{e}	64.98±0.33 ^{g}	11.23±0.45 ^{e}
G2 (Healthy+chitosan)	13.47±0.17 ^{d}	70.2±0.78 ^{e}	12.72±0.35 ^{c}
G3(Healthy+wheat bran	13.19±0.21 ^{de}	68.19±0.55 ^{f}	11.69±0.7 ^{de}
G3(Healthy+ chitosan+wheat bran	13.16±0.15 ^{e}	69.75±0.86 ^{e}	12.03±0.72 ^{d}
HF-HC control	23.63±0.5 ^{c}	109.96±1.56 ^{d}	19.88±0.62 ^{b}
HF-HC+chitosan	25.32±0.37 ^{a}	118.17±0.46 ^{a}	21.07±0.61 ^{a}
HF-HC+wheat bran	24.32±0.3 ^{b}	113.2±2.44 ^{c}	20.44±1.11 ^{ab}
HF- HC+ chitosan+Wheatbran	24.42±0.41 ^{b}	115.41±0.78 ^{b}	20.42±0.45 ^{ab}
L.S.D ($P<0.05$)	0.31	1.17	0. 67

Discussion:

The fat enriched diet is regarded as an important factor in the development of cardiac diseases since it leads to the development of hyperlipidemia, atherosclerosis and abnormal lipid metabolism (Onody *et al*., 2003 and Vijaimohan *et al*., 2006). In the present study, the effects of chitosan and wheat bran either alone or in combination on the alterations of lipid and lipoprotein profile in HF-HC rats were investigated. Jayasooriya *et al*. (2000), Barakat and Mahmoud (2011) reported that the rats fed with high cholesterol diet showed significant increase in body weight and liver weight, which leads to secondary complications clinically. In this study, the

Table 6: Effect of supplementing chitosan, wheat bran or the combination of two on serum MDA,blood GSH and GPx and liver NO in healthy and HF-HC rats

Groups	MDA (mg/dL)	GSH (mg/dL)	GPx (mg/dL)	NO
G1(control)	3.2 ±0.13^{e}	44.16 ±1.35 ^{e}	17.7 ± 0.16 ^{b}	60.74±1.17 ^{c}
G2 (Healthy+chitosan)	2.6 ±0.11 ^{g}	51.12 ±0.78 ^{a}	18.88 ± 0.06 ^{a}	66.53±0.66 ^{a}
G3(Healthy+wheat bran	2.9 ±0.07 ^{f}	45.87 ±0.78 ^{cd}	18.1 ± 0.55 ^{b}	62.1±0.8 ^{bc}
G3(Healthy+ chitosan+wheat bran	2.87 ±0.08 ^{f}	49.74 ±0.38 ^{b}	17.97 ±0.16 ^{b}	63.31±0.89 ^{b}
HF-HC control	7.83±0.2 ^{a}	34.59±1.9 ^{f}	11.73±0.55 ^{e}	33.48±1.15 ^{f}
HF-HC+chitosan	4.58±0.15 ^{d}	47.02±0.35 ^{c}	14.3±0.26 ^{c}	46.12±1.44 ^{d}
HF-HC+wheat bran	6.12±0.09 ^{b}	44.82±1.19^{de}	13.02±0.55 ^{d}	42.93±3.05 ^{e}
HF- HC+ chitosan+Wheatbran	5.41±0.1 ^{c}	45.18±1.83 ^{de}	13.17±0.6 ^{d}	44.29±1.33 ^{e}
L.S.D (P<0.05)	0.12	1.21	0.42	1.49

body and relative organs weight gain in hypercholesterolemic rats were decreased significantly upon treatment with chitosan or wheat bran or a combination of chitosan and wheat bran mixture. The hypolipidemic and antiatherogenic effects of chitosan and wheat bran may be responsible for the beneficial action of these fibers on body weight gain and liver weights. The results of Kondo and Osada, 1996 confirmed the results of this study who reported that male albino rats fed a semipurified diet supplemented with 5% chitosan for 31 days, the food intake and body weight gain are lower than control. These results suggest that among the dietary fibers used, chitosan has the most pronounced effect on the nutritional status of animals. The adverse effect on body weight was also observed in one-day-old broiler chickens fed a diet composed of 3g/kg of 89% deacetylated chitin (chitosan). The treated birds had significantly reduced body weights and feed intake compared to controls (Razdan *et al*., 1997). Furthermore Vuksan *et al*., 1999 reported that wheat fiber and protein for human consumption, has a laxative effect that is equivalent to or greater than that seen with standard wheat bran. In addition, it may have a favorable effect on serum lipids that requires confirmation by further studies.

Moreover (Gallaher *et al*., 2000) stated that the body and liver weights of the rats fed chitosan were significantly lower than those fed the control diet. The reduced rate of growth of the chitosan group was undoubtedly due to a reduced food intake. Our results are also agree with (Vijaimohan *et al*., 2006) who reported that body weight gain and liver weight of rats fed with high fat diet significantly increased as compared with rats fed normal basal diet. Makni *et al*., 2008 also reported that body weight gain differed significantly between hypercholesterolemic rats and normal control rats. This increase was probably due to the high cholesterol diet. Xia *et al*., 2011 reported that rats in the HF and CIS groups fed the same diets except for cellulose in the HF group being replaced with chitosan in the CIS group and had similar food intakes, but the body-weight gains of rats in the CIS groups were significantly lower than those in the HF group. Moreover, the retardation of body-weight gain in rats was obvious for a long time with chitosan treatment. This might indicate that chitosan could be used as weight-loss agent for both healthy and obese humans because of its binding of lipids in the gastrointestinal tract to reduce fat absorption (Shields *et al*., 2003).

Cholesterol- enriched diet resulted in a significant increase in total cholesterol, total lipids, phospholipids and triacylglycerol in plasma and liver and LDL-C levels, with decreased circulating HDL-C, thus providing a model for dietary hyperlipidemia (Makni *et al*., 2008) .The increase of lipid parameters has been shown to be a strong risk factor for coronary heart diseases in many populations (Makni *et al*., 2008).

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 (Jaganathan *et al*., 1974 and Vijaimohan *et al*., 2006). The metabolism of free and ester cholesterol are impaired in liver, spleen and thymus tissue and the rate of turnover was specifically decreased in all tissues of hyperlipidemic rats (Feoli *et al*., 2003 and Vijaimohan *et al*., 2006). The major clinical complications of hyperlipidemia are lipid deposition, mostly cholesterol esters and cholesterol (Wissler, 1991 and Vijaimohan *et al*., 2006) which is consistent with the present report.

Our results indicated that both chitosan and wheat bran used individually or in combination had a strong hypolipidemic, hypotriglyceridemic and Hypocholesterolemic effects in serum and liver of normocholesterolemic and hypercholesterolemic rats with a reduction of plasma LDL-C levels and an increase in HDL-C levels. Furthermore, the atherogenic index markedly decreased causing a significant reduction in LDL/HDL ratio in all groups fed diet supplemented with either chitosan or wheat bran. Our results are agree with Makni *et al*., 2008 who stated that the increase in HDL-C or HTR ratio is one of the most important criteria of anti-hypercholesterolemic agent. Zhang *et al*., 2008 suggested that the hypolipidemic activity of chitosan functions both in healthy and hypercholesterolemic rats. Chitosan significantly lowered total cholesterol and triacylglycerol levels in the plasma and liver and increased the fecal excretion of fat and cholesterol, which was consistent with previous reports (Sugano *et al*., 1980; Kanauchi *et al*., 1994). Furthermore, the reduction of lipid levels in plasma and liver may be because of the increased fecal lipid excretion. Also, the increased excretion of

fecal fat because of chitosan was consistent with our previous in vitro study (Zhou *et al*., 2006) Thus, the reduced absorption of cholesterol and fat was an important hypolipidemic action of chitosan. The plasma and liver total cholesterol and triacylglycerol levels in the hypercholesterolemic group fed chitosan were a little higher than those in the healthy fed chitosan group. This result might suggest that chitosan can effectively prevent the hypercholesterolemia with a high fat diet, and the longer the time of treatment the better the hypocholesterolemic effect. A study in human subjects suggests that chitosan could not significantly increase fecal fat excretion and cause weight loss (Gades and Stem, 2003; Zhang *et al*., 2008; Xia *et al*., 2011). But the duration of the study was short (12 days).

Two other human studies indicated that chitosan was efficacious in facilitating weight loss and reducing body fat in obese adults (Shields *et al*., 2003; Schiller *et al*., 2001; Zhang *et al*., 2008). These investigations lasted 8 and6 weeks. Therefore, longer-term trials are necessary to assess the efficacy of this agent in inducing weight loss.

Oxidative stress is defined as a condition in which cellular antioxidant defenses are insufficient to keep the reactive oxygen species (ROS) levels below a toxic threshold. When a oxidative stress condition is prevailed in the cells the most common ROS such as hydroxyl, superoxide, and nitric oxide act as electron acceptors from biomolecules including lipid, proteins and nucleic acids, leading to their oxidation. However, various molecules including chitosan derivatives are able to scavenge the radicals at different levels (Cho *et al*., 2011). In agreement with previous reports (Makni *et al*., 2008) observed a decrease in the activities of the antioxidant enzymes in hypercholesterolemic rats, compared to those of controls. Such decreases may be associated with the production of α-, β- unsaturated aldehydes during lipid peroxidation. These compounds have the ability to increase oxidative stress by promoting the cellular consumption of glutathione and by inactivating selenium-dependent glutathione peroxidase. Glutathione (GSH) serves as a substrate for the enzyme GPx, and it has been suggested that, through its activity, GSH protects plasma against oxidative damage. Moreover, the scavenging abilities of grafted chitosan derivatives were related to the number of active hydroxyl groups in the molecules (Li *et al*., 2002). On the other hand, according to the free radical theory, the amino groups in chitosan can react with free radicals to form stable macromolecules (Sun *et al*., 2003). Hence, the ROS and lipid peroxidation results obtained in this study are in accordance with the reported information. Since, the GA-g-chitosans have gallic acid residues in its molecules they showed profound activities than that of plain chitosan. Moreover, the concentration of GSH in most mammalian cells is relatively high and is continuously synthesized to maintain its level (Orrenius, 1994). The potential of GA-g-chitosans to maintain GSH at high levels could be of great importance to combat oxidative stress-induced toxicity in cells. Senevirathne *et al*., 2011 reported that chitosan increased catalase, glutathione peroxidase, and superoxide dismutase activities significantly ($p < 0.05$), suggesting that the scavenging of ROS by GA-g-chitosans may in part be related to increased activities of antioxidant enzymes. The cells possess an intricate network of defense mechanisms including antioxidant compounds such as GSH, and antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidases to neutralize excessive ROS accumulation. A major type of defense system in living tissues against oxidative damage is the use of antioxidant enzymes to convert ROS into non-toxic compounds and the tissue activities of those enzymes have been reported to be changed in response to the oxidative stress (Wispe *et al*., 1992; Jaruga *et al*., 1994). Senevirathne *et al*., 2011 added that chitosan reduced the oxidative stress via inhibition of ROS and lipid peroxidation as well as increment of antioxidant enzymes.

In conclusion, the data generated by this study demonstrated that chitosan is effective in lowering plasma and liver lipid levels in rats fed high-fat, high cholesterol diets and increasing fecal fat excretion. Moreover, it prevented and improved the hypercholesterolemia condition in rats fed the atherogenic diet. All of these results provide greater insight on the potential use of chitosan in humans as it acts against oxidative stress as well as increment of antioxidant enzymes. The hypolipidemic mechanism by which chitosan may be because of the reduced absorption of dietary fat and cholesterol

ACKNOWLEDGMENT

The author thanks Prof. Dr. Mona A. Sadek, Professor of Nutrition, Biochemistry and Nutrition Department, Women's college, Ain Shams University for her great help, guidance, and appropriate choice of the research topic.

REFERENCES

Allian, C., L. Poon, W. Richmond and P. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Barakat, L.A.A. and R.H. Mahmoud, 2011. The antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats. North American Journal of Medical Sciences, 3(9): 351-357.

Beutler, E., 1982. Red cell metabolism - A manual of biochemical methods, Beutler, E. ed. Grune and Stratton. New York, 137-140.

Burstein, M., H. Scholnik and R. Morfin, 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyphenols. J lipid Res., 11: 583-595.

Cho, Y. S., S.K. Kim, C.B. Ahn and J.Y. Je, 2011. Preparation, characterization, and antioxidant properties of gallic acid-grafted-chitosans. Carbohydrate Polymers, 83: 1617-1622.

Deuchi, K., O. Kanauchi, Y. Imasato and E. Kobayachi, 1994. Decreasing effect of chitosan on the apparent fat digestibility by rats fed on a high-fat diet. Biosci. Biotechnol. Biochem., 58: 1613-6.

Feoli, A., C. Roehrig, L. Rotta and *et al*., 2003. Serum and liver lipids in rats and chiks fed with diets containing different oils. Basic Nutrit Investig., 19: 789-793.

Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological chemistry, 226: 497-509.

Fossati, P. and L. Prencipe, 1982. Serum triglycerides determination calorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem., 28: 2077-2088.

Friedwald, W., R. Levy and D. Fredrichson, 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem., 226: 499-502.

Gades, M.D. and J.S. Stern, 2003. Chitosan supplementation and fecal fat excretion in men. Obes. Res., 11: 683-8.

Gallaher, C.M., J. Munion, R. Hesslink, J. Wise and D.D. Gallaher, 20003. Cholesterol reduction by glucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats. J. Nutr., 130: 2753-9.

Günzler, V.A., H. Kremers and L. Flohe, 1974. An improved coupled test procedure for glutathione peroxidase (EC 1.11.1.9.) in blood. Z. Klin. Chem. Biochem., 12: 444-448.

Jaganathan, S.N., W.E. Conor, W.H. Baker and A.K. Bahattacharya, 1974. The turnover of cholesterol in human atherosclerotic arteries. Journal of clinical investigation, 54(2): 366-370.

Jahromi, F., A.B. Ray and J.P.N. Chansouria, 1993. Antihyperlipidemic effect of flavonoids from pterocarpus marsupium. Journal of Naturnal products, 56: 989-994.

Jaruga, P., T.H. Zastawny, J. Skokowski, M. Dizdaroglu and R. Olinski, 1994. Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer. FEBS Letters, 341: 59–64.

Jayasooriya, A., N. Sakono, C. Yukizaki, M. Kawano, K. Yamamoto, N. Fukerda, 2000. Effect of Momordica charantia powder on serum glucose levels and various lipid parameters in rats fed with cholesterol enriched rats. J Ethnoparmacol., 72: 331-336.

Kahkonen, M.P., A.I. Hopia and M. Heinonen, 2001. Berry phenolics and their antoxidant activity.Journal of Agriculture and Food chemistry, 49: 4076-4082.

Kanauchi, O., K. Deuchi, Y. Imasato and E. Kobayashi, 1994. Increasing effect of a chitosan and ascorbic acid mixture on fecal dietary fat excretion. Biosci Biotechnol Biochem., 58: 1617-20.

Kondo, H. and A. Osada, 1996. Influence of dietary fiber on the bioavailability of zinc in rats. Biomed Environ Sci., 9: 204-208.

Lecumberri, E., L. Goya, R. Mateos, *et al*., 2007. A diet rich in dietary fiber from cocoa improves lipid profile and reduced malondialdehyde in hypercholesterolemic rats. Nutrition, 23: 332-341.

Li, W.J., X. Jiang, P.H. Xue and S.M. Chen, 2002. Inhibitory effects of chitosan on superoxide anion radicals and lipid free radicals. Chinese Scientific Bulletin, 11: 887–889.

Makni, M., H. Fetoui, N. Gargouri, H. Jaber, T. Boudawara and N. Zeghal, 2008. Hypolipidemic and hepatoprotective effects of flaxseed and pumpkin seed mixture in ω-3 and ω-6 fatty acids in hypercholesterolemic rats. Food Chem. Toxicol., 46: 3714-3720.

Mihara, M. and M. Uchiyama, 1978. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Analyt. Biochem., 86: 271–278.

Montgomery, H.A.C. and J.F. Dymock, 1961. Nitric oxide assay. Analyst, 86: 414.

Muzzarelli, R.A.A., 2000. Interactions of chitin, chitosan, Nlauryl chitosan and N-dimethylaminopropyl chitosan with olive oil. Carbohyd Polym., 43: 263-268.

Onody, A.M., C. Csonka, Z. Giricz and P. Ferdinandy, 2003. Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxidative fotmation in rat hearts. Cardiovascular Research, 58(3): 663-670.

Orrenius, S., 1994. Mechanisms of oxidative cell damage: An overview. In R. Paoletti, B. Samuelsson, A. L. Catapano, A. Poli, & M. Rinetti (Eds.), Oxidative process and antioxidants (pp. 53–71). New York: Raven Press.

Orrenius, S., 1994. Mechanisms of oxidative cell damage: An overview. In R. Paoletti, B. Samuelsson, A. L. Catapano, A. Poli, & M. Rinetti (Eds.), Oxidative process and antioxidants (pp. 53–71). New York: Raven Press.

Parejo, I., F. Viladomat, J. Bastida, A. Rosas-Romero, N. Flerrrlage, J. Burillo and C. Codina, 2002. Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. Journal of Agriculture and Food chemistry, 50: 6882-6890.

Razdan, A., D. Pettersson and J. Pettersson, 1997. Broiler chickens bod weights,feed intakes,plasma lipid and small –intestinal bile acids concentrations in response to feeding of chitosan and pectin. Br. J. Nutr., 78: 283-291.

Reeves, G., F.H. Nielsen and G.C. Fahmy, 1993. Purified diets for laboratory rodents: Final report of the American Institute of Nutrition on the reformation of rodent diet. J. Nutr., 123: 1939-1951.

Rockway, S. and M.A. Menard, 2000. Scientific review of chitosan: efficacy, applications and safety. Innov Food Technol., 9: 61–4.

Romero, A., K. West and T. Zern, 2002. Fernandez M. The seeds from plantago ovate lower plasma lipids by altering hepatic and bile acid metabolism in Guinea pigs. J Nutr., 132: 1194-1198.

Schiller, R.N., E. Barrager, A.G. Schauss and E.J. Nichols, 2001. A randomized, double-blind, placebo-controlled study examining the effects of a rapidly soluble chitosan dietary supplement on weight loss and body composition in overweight and mildly obese individuals. J. Am. Nutraceu Assoc., 4: 41-9.

Sciutto, A.M. and P. Colombo, 1995. Lipid-Lowering effect of chitosan dietary integrator and hypocaloric diet in obese subjects. Acta. Toxicol.Ther., 16: 215-230.

Senevirathne, M., Y. Jeon, Y. Kim, P. Park, W. Jung, C. Ahn and J. Je, 2011. Prevention of oxidative stress in Chang liver cells by gallic acid-grafted- chitosans. Carbohydrate Polymers.

Shields, K.M., N. Smock, C.E. McQueen and P.J. Bryant, 2003. Chitosan for weight loss and cholesterol management. Am J Health Syst Pharm., 60: 1310-12, 5-6.

Sugano, M., T. Fujikawa, Y. Hiratsuji, K. Nakashima, N. Fukuda, Y. Hasegawa, 1980. A novel use of chitosan as a hypocholesterolemic agent in rats.

Sun, T., P.X. Xu, Q. Liu, J. Xue and W.M. Xie, 2003. Graft copolymerization of methacrylic acid onto carboxymethyl chitosan. European Polymer Journal, 39: 189–192.

Takayama, M., S. Itoh, T. Nagasaki and I. Tanimizu, 1977. A new enzymatic method for determination of serum choline-containing phospholipids. Clin Chim Acta., 79: 93-98.

Tapola, N.S., M.L. Lyyra, R.M. Kolehmainen, E.S. Sarkkinen and A.G. Schauss, 2008. Safety aspects and cholesterol- lowering efficacy of chitosan tablets. Journal of American College of Nutrition, 27(1): 22-30.

Veneroni, G., F. Veneroni, S. Contos, S. Tripodi, M. De Bernardi, C. Guarino and M. Marletta, 1996. Effect of a new chitosan dietary integrator and hypocaloric diet on hyperlipidemia and overweight in obese patients. Acta Toxicol.Ther., 17: 53-70.

Venkateson, N., S. Devaraj and H. Devaraj, 2003. Increased binding of LDL and VLDL to apo B, E receptors of hepatic plasma membrane of rats treated with fibernat. Europ J Nutr., 42: 262-271.

Vijaimohan, K., M. Jainu, K. Sabitha, S. Subramanifam, C. Anandhan, C. Devi, 2006. Beneficial effects of alpha linolenic acid rich flaxseed oil on growth performance and hepatic cholesterol metabolism in high fat diet fed rats. Life Science, 79: 448-454.

Vuksan,V., D.J.A. Jenkins, E. Vidgen, T.P.P. Ransom, M.K. Ng, C.T. Culhane and D. O'Connor, 1999. A novel source of wheat fiber and protein: effects on fecal bulk and serum. Am J Clin Nutr., 69: 226–30.

Wispe, J.R., B.B. Warner, J.C. Clark, C.R. Dey, J. Neuman, *et al*., 1992. Human Mnsuperoxide dismutase in pulmonary epithelial cells of transgenie mice confers protection from oxygen injury. Journal of Biological Chemistry, 267: 23937–23941.

Wissler, R.W., 1991. Update on the pathogenrsis of atherosclerosis. American Journal of Medicine 91(1B), 3S-9S.

Xia, W., P. Lui, J. Zhang and J. Chen, 2011. Biological activities of chitosan and chitooligosaccharides. Food Hydrocolloids, 170-179.

Ylitalo, R., S. Lchtinen, E. Wuolijoki and P. Ylitalo, 2002. Cholesterol- lowering properties and safety of chitosan. Arzneimittelforschung, 52: 1-7.

Zhang, J., J. Liu, L. Li and W. Xia, 2008. Dietary chitosan improves hypercholesterolemia in rats fed high-fat diets, 28: 383-390.

Zhou, K., W. Xia, C. Zhang and L. Yu, 2006. In vitro binding of bile acids and triglycerides by selected chitosan preparations and their physicochemical properties. LWT-Food Sci Technol., 39: 1087-92.

Zollner, N. and K. Kirsch, 1962. Microdetermination of lipids by the Sulphophospho vanillin reaction. Z Ges Exp Med., 135: 545–561.