Australian Journal of Basic and Applied Sciences, 5(10): 1478-1488, 2011
ISSN 1991-8178

Effects of Chitosan and Wheat Bran on Serum Leptin, TNF-α, Lipid Profile and Oxidative Status in Animal Model of Non-Alcoholic Fatty Liver

Maha M. Mohamed

Home Economic Dept., Women's College, Ain Shams University, Asmaa Fahmy St.
Masr El-Gededa Cairo, Egypt.

Abstract: This study was carried out to investigate the capacity of chitosan and wheat bran to protect against high fat diet-induced hepatic steatosis. MATERIALS AND METHODS: Thirty six adult male Sprague-Dawley rats (average body weight of 120-150 g) were randomly assigned to six groups (n=6 rats/group) and fed on one of the following experimental diets: (i) basal diet, negative control; (ii) basal diet containing 5% chitosan, chitosan group; (iii) basal diet containing 5% wheat bran, wheat bran group; (iv) high fat diet (1% cholesterol + 7.5% corn oil + 15% beef tallow), positive control; (v) high fat diet containing 5% chitosan, high fat + chitosan; (vi) high fat diet containing 5% wheat bran, high fat + wheat bran. The trial lasted 6 weeks. RESULTS: High fat diet reduced food intake and increased body weight gain and liver weights. Also, High fat diet increased Hepatic fat deposition and elevated serum levels of triacylglycerol (TAG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) and decreased serum level of high-density lipoprotein cholesterol (HDL-C). This effect was associated with increased levels of serum tumor necrosis factor-α (TNF- α), leptin, alanine aminotransferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) and decreased serum levels of total proteins, albumin and globulin. Moreover, high fat diet elevated hepatic malondialdhyde and reduced the levels of hepatic reduced glutathione (GSH), erythrocytes copper, zinc superoxide dismutase (Cu, Zn-SOD) and plasma catalase. Dietary chitosan but not wheat bran supplementation significantly reduced high fat diet-induced hyperlipidemia and fat accumulation in liver. Furthermore, increased TNF-alpha and hyperleptinemia in high fat fed rats were significantly ameliorated by treatment with chitosan or wheat bran. Also, chitosan and wheat bran treatments ameliorated hepatic lipid peroxidation and antioxidant capacity. These effects were associated with improvement in liver function biomarkers. CONCLUSION: Chitosan has an advantage over wheat bran in exerting a significant hepatoprotective effect against non-alcoholic fatty liver. The hepatoprotective effect of chitosan might be ascribable to its antilipidemic, anti-inflammatory and/or antioxidant property.

Key words: Fatty liver; Chitosan; Wheat bran; Anti-oxidative effects; Anti-inflammatory effects.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of most common metabolic disorder characterized by fatty infiltration of the liver in the absence of alcohol consumption (Clark *et al*., 2003). Over-consumption of high calories of foods, particularly high fat diet, is crucial for the development of NAFLD. The pathophysiology of NAFLD has been conceptualized to be a two-stage process, consisting of fat accumulation in hepatocytes and consequent hepatic steatosis in the first stage, and hepatic injury or non-alcoholic steatohapatitis (NASH) in the second stage. Hepatic steatosis is generally benign, relatively non-aggressive and reversible. However, because hepatic steatosis can progress to fibrosis (in 20-40% of patients), cirrhosis (in 30% of patients) or hepatocellular carcinoma (Angulo, 2002 and Jiang & Torok, 2008) early prevention and treatment are essential. Regulation of excessive lipid synthesis and uptake is thought to be an effective intervention for NAFLD. Thus, lipid-lowering agents are promising pharmacological therapies for hepatic steatosis (Hong *et al.,* 2007 and Qin & Tian, 2010).

Chitosan, a cationic polysaccharide produced by the *N*-deacetylation of chitin under alkaline conditions, contains a linear sugar backbone, composed of β-1, 4-linked glucosamine units. Chitin is found in the shells of invertebrates such as shrimp and crabs (Hirano, 1996) and also found in the exoskeleton of arthropods and certain fungi (Furda 1983). Although chitosan is not derived from plants, it shares the characteristic with dietary fiber of being a polysaccharide that is indigestible by mammalian digestive enzymes (Gallaher *et al.,* 2000). Chitosan has been shown *in vitro* to bind and precipitate micellar lipids including bile salts, cholesterol and triacylglycerol (Nauss *et al*., 1983). Also, chitosan is reported to exhibit numerous health-related beneficial effects, including hypolipidemic, immunity regulation, anti-tumor, liver protection, anti-diabetic, antioxidant, anti-obesity, antibacterial and wound healing actions (Friedman and Juneja, 2010).

Corresponding Author: Maha M. Mohamed, Home Economic Dept., Women ˆs College, Ain Shams University, Asmaa Fahmy St. Masr El-Gededa Cairo, Egypt.
E-mail: Maha_nutrition@yahoo.com

Whole grain consumption has been linked to a lower risk of metabolic syndrome. The benefits of whole grain are in part related to the inclusion of the bran, rich in phenolic acids and fiber (Anson *et al.,* 2010). Cereal fibers are chiefly insoluble. Nevertheless, they have also been considered to be lipid-lowering agents although this effect is generally ascribed to soluble fibers (Arjmandi *et al*., 1992). Oat, rye and barley products have generally been found to be effective cholesterol-lowering agents (Bourdon *et al*., 1999), whereas the majority of studies have found no lipid reduction effect to wheat bran (Anderson *et al.,* 1988; Wanderson *et al.,* 1991 and Adam *et al*., 2001). However, there remain a significant number of studies where diets containing wheat bran have been associated with lower serum cholesterol or lipoprotein concentrations (Anderson *et al*., 1991; Lampe *et al.,* 1991 and Kashtan *et al*., 1992).

The present study aimed to evaluate the hypolipidemic, anti-inflammatory and antioxidant effects of chitosan and wheat bran against nutritional hepatic steatosis in rats.

MATERIALS AND METHODS

Materials:

Chitosan was prepared by deacetylation of chitin present in the shell of shrimp as described by Hussien & Abdeen (2008). Wheat bran was purchased from local market. The prepared chitosan and wheat bran were added to the basal diet at dose 5% according to Zhang *et al.,* (2008) and Abnous *et al.,* (2009), respectively. Kits for the biochemical measurements were purchased from Stanbio, Texas. Leptin and TNF-α enzyme immunoassay kits were purchased from SPI BIO, France. All other chemicals were of analytical grade.

Animals, Diets and Treatment:

Thirty-six adult male Sprague-Dawley albino rats (120-150 g) were obtained from Helwan farm of Serum and Vaccine, Cairo, Egypt. The animals were housed individually in metallic cages under healthy conditions. Water and basal diet were provided ad-libitum for one week as an adaptation period. Basal diet was based on AIN-93 recommendations (Reeves *et al.,* 1993). Following one week of acclimatization, rats were randomly divided into six groups (n = 6 rats / group) and fed on the following experimental diets (table 1):

(i) basal diet (negative control group)
(ii) basal diet containing 5% chitosan (chitosan group)
(iii) basal diet containing 5% wheat bran (wheat bran group)
(iv) high fat diet (15% tallow + 7.5% corn oil + 1% cholesterol) (positive control group)
(v) high fat diet + 5% chitosan (high fat + chitosan group)
(vi) high fat diet + 5% wheat bran (high fat + wheat bran group)

Table 1: Composition of experimental diets.

	Basal diet	Chitosan	Wheat bran	High fat	High fat+chitosan	High fat+wheat bran
	g \ 100 g diet					
Casein	17	17	17	17	17	17
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Cholin chloride	2	2	2	2	2	2
Vitamin mixture*	1	1	1	1	1	1
Mineral mixture*	4	4	4	4	4	4
Cholesterol	-	-	-	1	1	1
Corn oil	7.5	7.5	7.5	7.5	7.5	7.5
Beef tallow	-	-	-	15	15	15
Sucrose	5	5	5	5	5	5
Chitosan	-	5	-	-	5	-
Wheat bran	-	-	5	-	-	5
Cellulose	5	-	-	5	-	-
Starch	58.2	58.2	58.2	43.2	43.2	43.2

*AIN93 vitamin and mineral mixture (Reeves *et al*., 1993).

The animals were fed ad-libitum their different experimental diets for six weeks. Food intake was measured every two days and body weights were measured once every week. At the end of treatment period the animals were fasted overnight. Then, rats were sacrificed under diethyl ether anesthesia. The blood samples were collected from the portal vein in heparinized and non- heparinized centrifuge tubes. Serum and plasma were separated by centrifugation at 3000 r.p.m for 15 minutes. Serum and plasma aliquots were frozen at -20 ºC until further determination of other parameters. The erythrocytes were washed twice with cold saline and kept at -20 °C for Zn, Cu SOD estimation. Liver was removed, washed in cold saline, plotted in filter paper, weighed and used for the biochemical assays.

Biochemical Analysis:

Assay for Serum Lipids:

Concentrations of serum TAG, TC and HDL-C were determined enzymatically according to the colorimetric method of Fossati & Prencipe (1982), Allain, (1974) and Burstein *et al*. (1970), respectively, using Stanbio kits. Serum LDL-C value was calculated according to the equation of Friedwald *et al*., (1972) [LDL-C=TC-(HDL-C + TAG/5)].

Assay for Liver Lipid:

Lipids were extracted from the livers by the method of Bligh and Dyer, (1959). The concentrations of hepatic TC and TAG in lipid extract were measured enzymatically by use of commercial assay kits.

***Tumor Necrosis Factor-Alpha (TNF-Alpha) and Leptin*:**

Serum TNF-α and leptin levels were measured by various commercially available rat enzyme-linked immunosorbent assay (ELISA) kits. Specifically, for TNF-α levels the ALPCO Diagnostics ELISA kit and for leptin levels measurements the SPI ELISA kit was used.

Lipid Peroxidation (MDA) and Antioxidants:

Malondialdhyde (MDA) was measured colorimatrically at 535 and 520 nm, according to Mihara &Uchiyama (1978). Determination of GSH in liver is based on the method of Beutler *et al*. (1963). The determination of Cu, Zn-SOD activity in erythrocytes was according to Winterbourne *et al.* (1975). Catalase activity was estimated by following the decomposition of H_2O_2 directly by the decrease in extinction of hydrogen peroxide at 240 nm (Aebi *et al.*, 1972).

Liver Function Tests:

The levels of AST and ALT activities were assayed according to Henry *et al*., (1960) and ALP activity was assayed according Tietz *et al.,* (1983). Total proteins, albumin and total bilirubin were estimated by standard spectrophotometric methods according to Henry *et al*., (1974), Grant and Kachmar (1976) and Doumas *et al*., (1973), respectively.

Statistical Analysis:

The data are expressed as mean ± standard error of mean (mean ± SEM). The significant differences among groups were determined by one-way analysis of variance using the SPSS package program, version 11. The results were considered significant if the value of p was < 0.05, and Duncan's multiple range test was performed if differences were identified between groups (Duncan, 1955 and Bailey, 1994).

RESULTS AND DISCUSSION

Food Intake:

In the high fat-fed group, the food intake was significantly ($p < 0.05$) lower by about 21% than the negative control group. However, there was no significant difference in the food intake of the high fat + chitosan and high fat + wheat bran groups as compared with negative and positive controls. Similarly, no significant difference ($p > 0.05$) was found in the food intake of the chitosan and wheat bran groups when compared to the negative control group.

Body Weight Gain:

The high-fat diet caused significant ($p < 0.05$) increase (44.5%) in body weight gain compared with the control diet. Feeding high fat diets supplemented with chitosan significantly ($p < 0.05$) decreased body weight gain by 11.8% in the high fat + chitosan group compared with the positive control. However, wheat bran resulted in a small but not significant ($p > 0.05$) decrease in body weight gain compared with high fat group (Table 1). In the chitosan and wheat bran groups, no significant ($p > 0.05$) difference was found in the increase of body weights compared to negative control during the experimental period.

The Absolute and Relative Weights of Liver:

Both absolute and relative liver weights of high fat group were significantly ($p < 0.05$) higher by 40.3% and 26.7%, respectively, compared to the negative control. In the high fat + chitosan group the absolute weight of liver was significantly ($p < 0.05$) decreased by 12% as compared to high fat fed group. However, there was no significant ($p > 0.05$) difference in the absolute weight of liver in the group fed high fat + wheat bran as compared to positive control group. Meanwhile, the relative weight of liver was significantly ($p < 0.05$) decreased in both groups fed high fat diet supplemented with chitosan (13.6%) or wheat bran (9.8%).

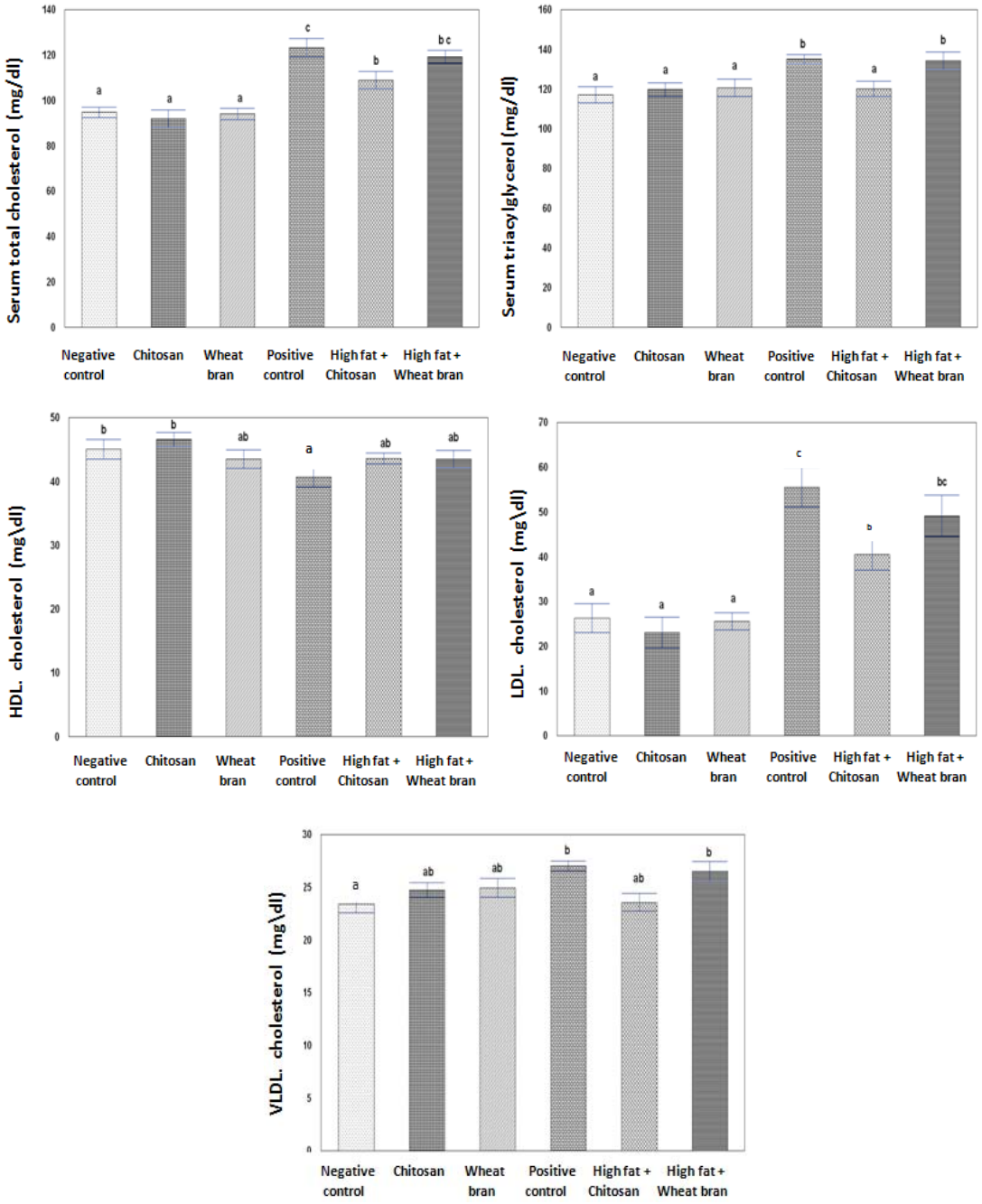

Fig. 1: Effect of chitosan and wheat bran on serum lipid profile in rats fed on basal diet or high fat diet The values are expressed as mean ± SEM (n = 6 rats/ group). The same letters means that there is no significant difference between groups. The different letters means that there is a significant difference between groups at $p<0.05$.

Serum Lipids Profile:

The high-fat diet significantly ($p < 0.05$) elevated the serum levels of TC (30.2%), TAG (15.2%), LDL-C (111.3%) and VLDL-C (15.3%) and decreased the serum level of HDL-C (9.5%) in the high fat group compared with the negative control group. Chitosan significantly ($p<0.05$) reduced serum TC (11.6%) and LDL-C levels (11.1%) in the high fat + chitosan group compared with the high fat group. Also, chitosan kept the levels of TAG, HDL-C and VLDL-C near normalcy in the high fat + chitosan group. However, wheat bran reduced but not significantly ($p > 0.05$) the levels of serum TC, TAG, LDL-C and VLDL-C in the high fat + wheat bran group compared with the high fat group. While, the level of HDL-C was nearly normalized in the high fat + wheat bran group.

Table 2: Effects of chitosan and wheat bran on body weight gain, Food intake and Liver weights (absolute and relative) in rats fed basal or high fat diet.

Parameters Groups	Food intake (g\ rat\ day)	Body weight gain (g\ 6 weeks)	Liver weight (g)	Relative weight of liver (g%)
Negative control	10.9±1.8^{b}	63.08±6.6^{a}	7.94±0.44^{a}	4.34±0.087^{b}
Chitosan	11.5±1.2^{b}	63.8±3.8^{a}	8.7±0.25^{a}	3.81±0.081^{a}
Wheat bran	10.8±1.05^{b}	60.66±6.3^{a}	8.1±0.42^{a}	3.7±0.108^{a}
High fat (Positive control)	8.6±1.02^{a}	91.2±5.3^{c}	11.14±0.46^{c}	5.5±0.22^{d}
High fat + Chitosan	9.1±0.98^{ab}	80.4±5.07^{b}	9.8±0.45^{b}	4.75±0.147^{c}
High fat +Wheat bran	9.14±0.79^{ab}	87.7±5.01^{bc}	10.63±0.35^{c}	4.96±0.1^{c}

The values are expressed as mean ± SEM (n= 6 rats/ group). The same letters means that there is no significant difference between groups. The different letters means that there is a significant difference between groups at $p<0.05$

Hepatic Lipids:

Hepatic TC and TAG levels were significantly ($p < 0.05$) elevated by 13% and 25.3%, respectively, in high fat-fed group as compared to negative control. Dietary chitosan normalized hepatic TC and TAG and significantly ($p < 0.05$) reduced their levels by 9.5% and 9.2%, respectively, in high fat + chitosan fed rats as compared to positive control. However, high fat + wheat bran group showed no significant change ($p>0.05$) in the levels of hepatic TC and TAG as compared to positive control. Also, in basal diet fed rats, chitosan and wheat bran had no influence on hepatic TC or TAG as compared to negative control.

Tumor Necrosis Factor-Alpha (TNF-Alpha) and Leptin:

Serum TNF-alpha and leptin levels were significantly ($p < 0.05$) elevated by 47.6% and 60.1% in the high fat group compared with the negative control group. Chitosan and wheat bran partly prevented the high-fat diet-induced increase in serum levels of TNF-alpha (25.2% and 28.3%, respectively) and leptin (21.2% and 12.9%, respectively) in the high fat + chitosan or high fat +wheat bran groups as compared with positive control.

Lipid Peroxidation (MDA) and Antioxidant Capacity:

The hepatic MDA level was significantly ($p < 0.05$) higher by 16.5% and hepatic GSH was significantly ($p < 0.05$) lower by 21.3% in the high fat group compared with the negative control group. Additionally, erythrocytes levels of SOD and plasma catalase were significantly ($p < 0.05$) lower by 42.1% and 15.7%, respectively, in the high fat group compared with the negative control group. Supplementation of the high fat diet with chitosan or wheat bran normalized the elevated level of hepatic MDA and the depressed levels of hepatic GSH. Also, dietary chitosan or wheat bran significantly ($p < 0.05$) increased the lowered levels of erythrocytes SOD (59% and 33.6%, respectively) and plasma catalase (14.2% and 13.2%, respectively) as compared with positive control.

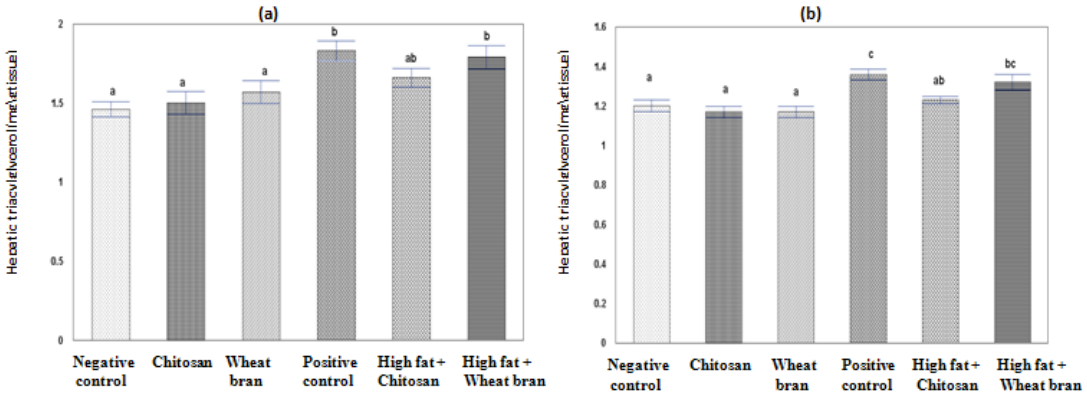

Fig. 2: Effect of chitosan and wheat bran on hepatic total cholesterol (a) and triacylglycerols (b) in rats fed on basal diet or high fat diet. The values are expressed as mean ± SEM (n= 6 rats/ group). The same letters means that there is no significant difference between groups. The different letters means that there is a significant difference between groups at $p < 0.05$.

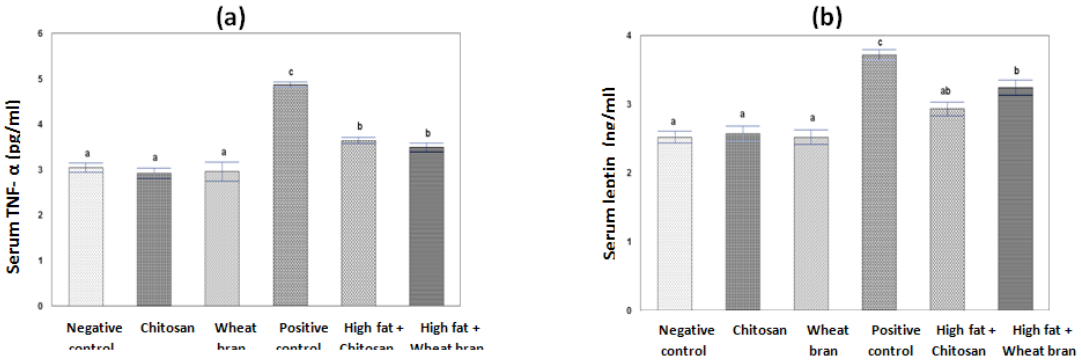

Fig. 3: Effect of chitosan and wheat bran on serum tumor necrosis factor-α (TNF- α) (a) and leptin (b) in rats fed on basal diet or high fat diet. The values are expressed as mean ± SEM (n = 6 rats/ group). The same letters means that there is no significant difference between groups. The different letters means that there is a significant difference between groups at $p < 0.05$.

Table 3: Effects of chitosan and wheat bran on hepatic MDA and GSH and activities of plasma catalase and erythrocytes SOD in rats fed on basal or high fat diet.

Parameters Groups	Hepatic MDA (nmol\g)	Hepatic GSH (mg\g)	Erythrocytes Cu-Zn SOD (U\ml)	Plasma Catalase (U\ml)
Negative control	51.27±2.3^{a}	15.33±0.42^{b}	299.17±5.38^{c}	84.2±1.97^{b}
Chitosan	49.6±1.55^{a}	15.08±0.39^{b}	254.5±20.6^{bc}	82.9±1.13^{b}
Wheat bran	52.76±2.4^{a}	14.36±0.54^{b}	285±16.4^{bc}	82.1±1.6^{b}
High fat (Positive control)	59.78±3.2^{b}	12.05±0.5^{a}	172.95±9.97^{a}	70.9±2.52^{a}
High fat + Chitosan	55.06±1.7^{ab}	15.2±0.48^{b}	275.05±30.1^{bc}	81±1.45^{b}
High fat +Wheat bran	54.96±2.3^{ab}	14.25±0.38^{b}	231.08±29.6^{bc}	80.3±0.72^{b}

The values are expressed as mean ± SEM (n= 6 rats/ group). The same letters means that there is no significant difference between groups. The different letters means that there is a significant difference between groups at $p<0.05$.

Table 4: Effects of chitosan and wheat bran on liver function tests in rats fed on basal and high fat diets.

Groups Parameters	Negative control	Chitosan	Wheat bran	High fat (Positive control)	High fat + chitosan	High fat + wheat bran
AST (U/L)	24.3±0.422^{a}	23.4±1.6^{a}	24.33±1.33^{a}	28.6±1.08^{b}	25.6±1.45^{a}	26.4±0.63^{ab}
ALT (U/L)	66.67±3.48^{a}	65.67±2.06^{a}	68.67±2.8^{a}	82.5±2.9^{b}	72.83±4.5^{a}	81.1±6.18^{b}
ALP (U/L)	80.45±10.3^{a}	83±7.02^{a}	76.5±5.3^{a}	115.2±6.13^{b}	96.03±4.2^{a}	108±4.4^{b}
Total bilirubin (mg/dl)	0.34±0.02^{a}	0.34±0.014^{a}	0.34±0.138^{a}	0.376±0.15^{a}	0.35±0.01^{a}	0.35±0.011^{a}
Direct bililrubin (mg/dl)	0.163±0.01^{a}	0.163±0.005^{a}	0.162±0.01^{a}	0.16±0.004^{a}	0.17±0.004^{a}	0.16±0.007^{a}
Indirect bilirubin (mg/dl)	0.183±0.02^{a}	0.177±0.015^{a}	0.182±0.01^{a}	0.181±0.013^{a}	0.17±0.013^{a}	0.19±0.009^{a}
Total protein (mg/dl)	7.58±0.27^{b}	7.51±0.27^{b}	7.5±0.25^{b}	6.4±0.51^{a}	7.48±0.17^{b}	7.5±0.36^{b}
Albumin (mg/dl)	4.43±0.28^{b}	4.05±0.25^{b}	4.2±0.33^{b}	2.95±0.25^{a}	3.96±0.21^{b}	4.5±0.145^{b}
Globulin (mg/dl)	2.95±0.14^{a}	3.49±0.23^{a}	3.41±0.27^{a}	3.5±0.42^{a}	3.2±0.33^{a}	3.28±0.17^{a}
A\G ratio	1.5±0.13^{b}	1.14±0.12^{ab}	1.29±0.18^{b}	0.86±0.067^{a}	1.32±0.16^{b}	1.37±0.07^{b}

The values are expressed as mean ± SEM (n= 6 rats/ group). The same letters means that there is no significant difference between groups. The different letters means that there is a significant difference between groups at $p<0.05$.

Liver Function Parameters:

Serum AST, ALT and ALP activities were significantly ($p < 0.05$) elevated by17.6%, 23.7% and 43.19%, respectively, in high fat fed group as compared to negative control. Animals fed on high fat diet supplemented with chitosan showed significant reduction in the activities of AST, ALT and ALP as compared to positive control. Meanwhile, the high fat diet supplemented with wheat bran reduced the activities of these enzymes but not significantly ($p > 0.05$). Serum total, direct and indirect bilirubin showed no significant ($p > 0.05$) changes between groups. However, serum total proteins and albumin were significantly ($p < 0.05$) reduced by 15.5% and 33%, respectively, in the high fat fed group compared with the negative control. The reduced serum total proteins and albumin were significantly ($p < 0.05$) increased by supplementation of the high fat diet with chitosan or wheat bran compared with the positive control.

Discussion:

In this study, we compared the efficacy of dietary chitosan or wheat bran treatment on the high fat diet-induced hepatic steatosis and hyperlipidemia in rats.

The results of the present study showed that food intake was significantly decreased in high fat fed rats, consistent with previous reports in this model of rats (Yalniz *et al*., 2007 and Zhang *et al*., 2010). The reason may be that a high fat diet may induce anorexia in rats (Tuomisto *et al*., 1999). Also, the body weight gain was significantly increased in the high fat-fed rats in spite of the decrease in the food intake. Schouten *et al*., (1985) explained this effect by the higher fat content and thus higher energy density of the high fat diet. Dietary chitosan treatment for 6 weeks significantly inhibited the high fat diet-induced increase in body weight gain, liver weights (absolute and relative) in the high fat fed rats. However, wheat bran consumption resulted in insignificant decrease in the body weights and the absolute weights of liver. This is in conformity with the results of previous studies in which chitosan limited the body-weight gain of adult rats (Deuchi *et al.*, 1995; Li *et al*., 2007 and Liu *et al*., 2008).

Furthermore, dietary chitosan or wheat bran mitigated the high fat diet-induced hyperlipidemia, and hepatic steatosis, which was associated with the improvement of liver function. However chitosan has an advantage over wheat bran in lowering serum and hepatic lipids. Previous studies documented that insoluble fibers such as wheat bran or cellulose do not have hypolipidemic effects (Anderson *et al.,* 1988; Wright *et al.,* 1990 and Anderson *et al.,* 1990). Nevertheless, many studies over the last three decades have reported that wheat fiber may lower serum lipids (Anderson *et al*., 1991; Lampe *et al.,* 1991 and Kashtan *et al*., 1992). Jenkins et al. (1999) suggested that wheat-fiber diets may be associated with increased cereal proteins and this may result in lower serum triglycerides.

Many studies showed that chitosan can lower plasma and liver TAG as well as TC levels (Maezaki *et al.*, 1993 and Cho *et al*., 1998) exhibiting hypocholesterolemic and hypolipidemic effects. It has been reported that chitosan has potent fat-binding capacity *in vitro* (Zhou *et al*., 2006). In addition, chitosan was also shown to increase fecal-neutral-steroid and bile-acid excretion in rats (Cho *et al*., 1998 and Gallaher *et al.* 2000) and lower the postprandial plasma TAG level in broiler chickens (Razdan *et al.,* 1994). Further, Nauss *et al.,* (1983) reported that *in vitro*, chitosan could bind micellar lipids in substantial amounts. These authors also indicated that chitosan could bind the microemulsions of lipids that occur within the small intestine after a fat-containing meal causing reduction in the absorption of all components of a micelle, *i.e*., bile acids, cholesterol, monoglycerides and fatty acids. Deuchi *et al*., (1994), however, proposed a quite different mechanism. They suggested that chitosan is dissolved in the stomach by gastric acid and subsequently mixes with dietary fat to form a chitosan-fat complex. This complex was hypothesized to gel in the small intestine, entrapping the fat and thereby preventing lipolysis, with subsequent excretion of the undigested fat, including cholesterol. Yet another explanation is provided by Han *et al*., (1999) who reported that, *in vitro*, chitosan inhibits pancreatic lipase activity. Inhibition of pancreatic lipase within the small intestine would lead to accumulation of a lipid emulsion. In the presence of substantial amounts of unabsorbed lipid within the small intestine, cholesterol will partition into the lipid phase (Jandacek, 1982), leading to greater excretion of cholesterol.

Leptin is exclusively secreted by adipocytes in proportion to their triglyceride stores, and circulating leptin levels are correlated with the extent of obesity (Maffei *et al.,* 1995 and Havel, 2000). It regulates energy expenditure and food intake through the central nervous system (Matysková *et al*., 2010). Obese subjects have higher leptin levels in comparison with normal-weight or lean subjects. Therefore, leptin is used as an indicator of obesity *in vivo* and *in vitro* (Matsubara *et al.,* 2002). In the present study, chitosan and wheat bran supplementation significantly reduced serum level of leptin, which was concomitant with the observed weight changes. The reduced levels of leptin in the serum could be a result of the observed weight reduction by chitosan and wheat bran and subsequent improvements of its sensitivity. The results of the present study are similar to the results of previous studies in which dietary supplementation with chitosan derived from mushrooms changes adipocytokine profile in diet-induced obese mice (Neyrinck *et al.,* 2009 and Liu *et al.,* 2011). Also Chuang *et al.* (2011) concluded that wheat bran intake was associated with a decreased level of leptin and pro-inflammatory cytokines.

The results of the current study showed that the pro-inflammatory cytokine TNF-α was significantly elevated in the serum of high fat diet fed rats. TNF-α inhibits the propagation of insulin receptor-initiated signals in hepatocytes and is known to promote insulin resistance (IR) and steatohepatitis in ob/ob mice and NAFLD patients (Crespo *et al*., 2001). IR plays a crucial role in NAFLD, fat accumulation in the liver and IR cause and potentiate each other, creating a vicious cycle of metabolic dysfunction, resulting in the development and progression of NAFLD (Gwozdziewiczová *et al*., 2005). TNF-α not only mediates the early stages of fatty liver disease, but also the transition to advanced stages of liver damage (Tilg and Diehl, 2000). In the present study, dietary chitosan and wheat bran lowered the serum level of TNF- α in the high fat fed rats. A number of studies have found anti-inflammatory activity of chitosan and its derivatives. Kim *et al*., (2002) reported that high molecular weight water soluble chitosan inhibits the production of pro-inflammatory cytokine, interleukin-1β and TNF-α, in human astrocytoma cells activated by Aβ peptide 25-35. Another study conducted by Khodagholi *et al*. (2010) showed that chitosan exerts anti-neuroinflammatory action by upregulation of heat shock protein 70 (Hsp-70) and inhibits the activation of nuclear factor kappa B (NF-κB).

Along with cytokines, oxidative stress also plays an important role in the second stage of NAFLD, mediating the progression of hepatic steatosis to NASH. In the present study, we found that the high-fat diet elevated the hepatic MDA level, and decreased the levels of antioxidants such as hepatic GSH and erythrocytes SOD and plasma CAT. Liver diseases associated with hepatic fat accumulation could amplify the production of reactive oxygen species because liver fat may serve as a substrate for lipid peroxidation. Elevated lipid peroxidation leads to increased MDA generation in NAFLD (Bujanda *et al.,* 2008). Moreover, oxidative stress probably plays a role in the progression of liver injury as MDA can stimulate the production of cytokines that participate in the activation of spindle cells and fibrogenesis (Sariçam *et al.,* 2005 and Bujanda *et al*., 2008). At the same time, the low levels of SOD, GSH and CAT in animal models and patients with NAFLD suggest their increased utilization because of enhanced oxidative stress.

Dietary supplementation with chitosan and wheat bran reversed the changes in antioxidant activities induced by the high-fat diet and ultimately improved oxidative status; which constitute another mechanism by which chitosan and wheat bran prevent fatty liver. Anraku *et al*. (2010) suggested that chitosan might reduce certain levels of pro-oxidants such as cholesterol and uremic toxins in the gastrointestinal tract, thereby inhibiting the subsequent development of oxidative stress in the systemic circulation. The antioxidant properties of wheat bran may be related to the antioxidant components which present in the bran such as phenolic acids, lignans and phytoestrogens (Slavin, 2004).

The elevated levels of aminotransferases and alkaline phosphatase are the result of leakage from damaged cells, and are used as markers of liver injury, particularly for NAFLD (Giannini *et al.,* 2005). Abnormal aminotransferase activity was noted in rats fed the high fat diet, and was reversed by chitosan feeding. These results provide further evidence that chitosan could improve hepatic steatosis in rats.

Collectively, the results of the present study demonstrated that supplementation with chitosan might be effective in the protection against hepatic steatosis by control of dyslipidemia, anti-inflammatory effects as well as anti-oxidation properties. Also, the data of the current study showed that wheat bran has anti-anflammatory and antioxidant properties, however, chitosan has a better hypolipidemic effect than chitosan. Therefore, the result of this study support the notion that dietary control and personal lifestyle modifications are critical for the control of hyperlipidemia and associated NAFLD.

REFERENCES

Abnous, K., S.P. Brooks, J. Kwan, F. Matias, J. Green-Johnson, L.B. Selinger, M. Thomas and M. Kalmokoff, 2009. Diets enriched in oat bran or wheat bran temporally and differentially alter the composition of the fecal community of rats. J Nutr., 139: 2024-31.

Adam, A., M. Levrat-Verny, H.W. Lopez, M. Leuillet, C. Demign and C. Re´me´ sy, 2001. Whole wheat and triticale flours with differing viscosities stimulate cecal fermentations and lower plasma and hepatic lipids in rats. J Nutr., 131: 1770-6.

Aebi, H., 1972. Catalase In: Methods of enzymatic analysis, (Hu, B. ed.). Academic Press: New York; 673-684.

Allain, C.C., 1974. Quantitative-enzymatic colorimetric determination of total and HDL cholesterol in serum or plasma. Clin. Chem., 20: 470.

Anderson, J.C., N.H. Gilinsky, D.A. Dee A Deakins, S.F. Smith, D.S. Neal, D.W. Dillon and P.R. Oeltgen, 1991. Lipid responses of hypercholesterolemic men to oat-bran and wheat-bran intake. Am J Clin Nutr., 54: 678-83.

Anderson, J.W., S.R. Bridges, S.F. Smith, D.W. Dillon and D.A. Deakins, 1989. Oat bran increases serum acetate of hypercholesterolemic men. Clin Res., 37: 93.

Anderson, J.W., D.A. Deakins and S.R. Bridges, 1990. Soluble fiber hypocholesterolemic effects and proposed mechanisms. In: Dietary fiber chemistry, physiology, and health effects (Kritchevsky, D., Bonfield, C. Anderson, J.W. *eds*.) New York: Plenum Press, pp: 339-63.

Anderson, J.W., N. Zettwoch, T. Feldman, J. Tietyen-Clark, P. Oeltgen and C.W. Bishop, 1988. Cholesterol-lowering effects of psyllium hydrophilic mucilloid for hypercholesterolemic men. Arch Intern Med., l48: 292-6.

Angulo, P., 2002. Nonalcoholic fatty liver disease. N Engl J Med., 346: 1221-31.

Anraku, M., A. Michihara, T. Yasufuku, K. Akasaki, D. Tsuchiya, H. Nishio, T. Maruyama, M. Otagiri, Y. Maezaki, Y. Kondo and H. Tomida, 2010. The antioxidative and antilipidemic effects of different molecular weight chitosans in metabolic syndrome model rats. Biol. Pharm. Bull., 33: 1994-1998.

Anson, N.M., A.M. Aura, E. Selinheimo, I. Mattila, K. Poutanen, R. van den Berg, R. Havenaar, A. Bast and G.R.Haenen, 2010. Bioprocessing of wheat bran in whole wheat bread increases the bioavailability of phenolic acids in men and exerts antiinflammatory effects ex vivo. J Nutr., 141: 137-43.

Arjmandi, B.H., J. Ahn, S. Nathani and R.D. Reeves, 1992. Dietary soluble fiber and cholesterol affect serum cholesterol concentration, hepatic portal venous short-chain fatty acid concentrations and fecal sterol excretion in rats. J Nutr., 122: 246-53.

Bailey, N.T.J., 1994. Statistical methods in biology.3rd ed. Cambridge University press (Uk).

Beutler, E., O. Duron and B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification concentration. J. Biochem. Physiol., 37: 911.

Bourdon, I., W. Yokoyama, P. Davis, C. Hudson, R. Backus, D. Richter, B. Knuckles and B.O. Schneeman, 1999. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with beta-glucan. Am. J. Clin. Nutr., 69: 55-63.

Bujanda, L., E. Hijona, M. Larzabal, M. Beraza, P. Aldazabal, N. García-Urkia, C. Sarasqueta, A. Cosme, B. Irastorza, A. González and J.I. Arenas, 2008. Resveratrol inhibits nonalcoholic fatty liver disease in rats. BMC Gastroenterol, 8:40.

Burstein, M., H.R. scholnick and R. Morfin, 1970. Rapid method for isolation of lipoproteins from human serum by precipitation with polyanions. Lipid Res., 11: 583-95.

Cho, Y.I., H.K. No. and S.P. Meyers, (1998): Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. J Agric Food Chem., 46: 3839-43.

Chuang, S.C., R. Vermeulen, M.T. Sharabiani, C. Sacerdote, S.H. Fatemeh, F. Berrino, V. Krogh, D. Palli, S. Panico, R. Tumino, T.J. Athersuch and P. Vineis, 2011. The intake of grain fibers modulates cytokine levels in blood. Biomarkers., 16: 504-10.

Clark, J.M., F.L. Brancati and A.M. Diehl, 2003. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol., 98: 960-67.

Crespo, J., A. Cayón, P. Fernández-Gil, M. Hernández-Guerra, M. Mayorga, A. Domínguez Díez, J.C. Fernández-Escalante and F. Pons-Romero, 2001. Gene expression of tumor necrosis factor-a and TNF-receptors, p55 and p75 in non-alcoholic steatohepatitis patients. Hepatology., 34: 1158-63.

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

Deuchi, K., O. Kanauchi, M. Shizukuish and E. Kobayashi, 1995. Continuous and massive intake of chitosan affects mineral and fat–soluble vitamin status in rats fed on a high–fat diet. Biosci. Biotech. Biochem.,59: 1211-6.

Doumas, B.T., B.W. Perry, E.A. Sasse and Jr.J.V. Straumjord, 1973. Bilirubin-automatic, Jendrassik-Grof method. Clin. Chem., 19: 984-93.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics., 15: 1-42.

Fossati, P. and L. Prencipe, 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28: 2077-80.

Friedewald, W.T., R.I. Levy and D.S. Frerickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Friedman, M. and V.K. Juneja, 2010. Review of antimicrobial and antioxidative activities of chitosans in food. J Food Prot., 73: 1737-61.

Furda, I., 1983. Aminopolysaccharides—their potential as dietary fiber. In: Unconventional Sources of Dietary Fiber (Furda, I., ed.). ACS Symposium Series., 214: 105-22.

Gallaher, C.M., J. Munion, Jr. R. Hesslink, J. Wise and D.D. Gallaher, 2000. 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.

Giannini, E.G., R. Testa and V. Savarino, 2005. Liver enzyme alteration: A guide for clinicians. Can Med Assoc J., 172: 367-79.

Grant, G.H. and J.F. Kachmar, 1976. Fundamentals of clinical chemistry. (Tietz, N.W. ed.) W.B. Saunders CO. Philadelphia.

Gwozdziewiczová, S., R. Lichnovská, R.B. Yahia, R. Chlup and J. Hřebíček, 2005. TNF alpha in the development of insulin resistance and other disorders in metabolic syndrome. Biomed Papers., 149: 109-17.

Han, L.K., Y. Kimura and H. Okuda, 1999. Reduction in fat storage during chitin-chitosan treatment in mice fed a high-fat diet. Int. J. Obes. Relat. Metab. Disord., 23: 174-9.

Havel, P.J., 2000. Role of adipose tissue in body-weight regulation: Mechanisms regulating leptin production and energy balance. Proc. Nutr. Soc., 59: 359-371.

Henry, R.J., D.C. Cannon and J.W. Winkelman, 1974. Clinical chemistry principles and techniques (Harper and Row), 16-40.

Henry, R.J., N. Chiamori, O.J. Golub and S. Berkman, 1960. Revised spectrophotometric methods for the determination of GOT, GPT and LDH. Am. J. Clin. Path., 34: 381-398.

Hirano, S., 1996. Chitin biotechnology applications. Biotechnol A Rev., 2: 237-258.

Hong, X.Z., L.D. Li and L.M. Wu, 2007. Effects of fenofibrate and xuezhikang on high fat diet-induced non-alcoholic fatty liver disease. Clin Exp Pharmacol Physiol., 34: 27-35.

Hussien, M.H.M. and Z. Abdeen, 2008. Characteriz\tion and application of extracted natural polymer fiber (aminopolysaccharide) as weight reducing agent. The 3rd international conference of chemical industries. National Research Center, Cairo, Egypt.

Jandacek, R.J., 1982. The effect of nonabsorbable lipids on the intestinal absorption of lipophiles. Drug Metab. Rev., 13: 695-714.

Jenkins, D.J.A., C.W.C. Kendall, V. Vuksan, L.S.A. Augustin, C. Mehling, T. Parker, E. Vidgen, B. Lee, D. Faulkner, H. Seyler, R. Josse, L.A. Leiter, P.W. Connelly and V. Fulgoni, 1999. Effect of Wheat Bran on Serum Lipids: Influence of Particle Size and Wheat Protein. J Amer. Coll. Nutr., 18: 159-165.

Jiang, J. and N. Torok, 2008. Nonalcoholic steatohepatitis and the metabolic syndrome. Metab Syndr Relat Disord., 6: 1-7.

Kashtan, H., H.S. Stern, D.J. Jenkins, A.L. Jenkins, K. Hay, N. Marcon, S. Minkin and W.R. Bruce, 1992. Wheat-bran and oat-bran supplements' effects on blood lipids and lipoproteins. Am J Clin Nutr., 55: 976-80.

Khodagholi, F., B. Eftekharzadeh, N. Maghsoudi and P. Rezaei, 2010. Chitosan prevents oxidative stress-induced amyloid β formation and cytotoxicity in NT2 neurons: involvement of transcription factors Nrf2 and NF-κB. *Mol.* Cell. Biochem., 337: 39-51.

Kim, M.S., M.J. Sung, S.B. Seo, S.J. Yoo, W.K. Lim and H.M. Kim, 2002. Water-soluble chitosan inhibits the production of pro-inflammatory cytokine in human astrocytoma cells activated by amyloid [beta] peptide and interleukin-1 [beta]. Neurosci. Lett., 321: 105-9.

Lampe, J.W., J.L. Slavin, K.S. Baglien, W.O. Thompson, W.C. Duane, J.H. Zavoral, 1991. Serum lipid and fecal bile acid changes with cereal, vegetable, and sugar-beet fiber feeding. Am J Clin Nutr., 53: 1235-41.

Li, L., J.L. Zhang, J.N. Liu and W.S. Xia, 2007. Effects of chitosan on serum lipid and fat liver. Chin. J. Mar. Drugs., 26: 7-9.

Liu, J.N., J.L. Zhang and W.S. Xia, 2008. Hypocholesterolemic effects of different chitosan samples *in vitro* and *in vivo*. Food Chem., 107: 419-25.

Liu, X., F. Yang, T. Song, A. Zeng, Q. Wang, Z. Sun and J. Shen, 2011. Therapeutic effect of carboxymethylated and quanternized chitosan on insulin resistance in high-fat-diet-induced rats and 3T3-L1 adipocytes. J Biomater Sci Polym Ed. 8. [Epub ahead of print].

Maezaki, Y., K. Tsuji, Y. Nakagawa, Y. Kawai, M. Akimoto and T. Tsugita, 1993. Hypochloesterolemic effect of chitosan in adult males. Biosci. Biotech. Biochem., 57: 1439-44.

Maffei, M., J. Halaas, E. Ravussin, R.E. Pratley, G.H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan, P.A. Kern and J.M. Friedman, 1995. Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med., 1: 1155-1161.

Matsubara, M., S.S. Maruoka and S. Katayose, 2002. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. Eur J Endocrinol., 147: 173-80.

Matysková, R., B. Zelezná, J. Maixnerová, D. Koutová, M. Haluzík and L. Maletínská, 2010. Estradiol supplementation helps overcome central leptin resistance of ovariectomized mice on a high fat diet Horm Metab Res., 42: 182-6.

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

Nauss, J.L., J.L. Thompson and J. Nagyvary, 1983. The binding of micellar lipids to chitosan. Lipids, 18: 714-9.

Neyrinck, A.M., L.B. Bindels, F. De Backer, B.D. Pachikian, P.D. Cani and N.M. Delzenne, 2009. Dietary supplementation with chitosan derived from mushrooms changes adipocytokine profile in diet-induced obese mice, a phenomenon linked to its lipid-lowering action. Int Immunopharmacol., 9: 767-73.

Qin, Y. and Y. Tian, 2010. Preventive effects of chronic exogenous growth hormone levels on diet-induced hepatic steatosis in rats. Lipids in Health and Disease., 9:78.

Razdan, A. and D. Pettersson, 1994. Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens. Br. J. Nutr.,72: 277-88.

Reeves, P.G., F.H. Nielsen and G.C.Jr. Fahey, 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN 76A rodent diet. J Nutr., 123: 1939-51.

Sariçam, T., B. Kircali and T. Köken, 2005. Assessment of lipid peroxidation and antioxidant capacity in non-alcoholic fatty liver disease. Turk J Gastroenterol., 16: 65-70.

Schouten, J.A., A.C. Beynen, C. Popp-Snijders and C. Mulder, 1985. Effect of hypercholesterolemic diet on the fatty acid composition of rat erythrocyte phospholipids.. Nutr. Rep. Inter., 31: 229-236.

Slavin, J., 2004. Whole grains and human health. Nutr Res Rev., 17: 99-110.

Tietz, N.W., D. Rinker, L.M. Shaw, 1983. IFCC method for alkaline phosphatase. J Clin. Chim. Clin. Biochem., 21: 731-48.

Tilg, H. and A.M. Diehl, 2000. Cytokines in alcoholic and nonalcoholic steatohepatitis. N Engl J Med., 343: 1467-75.

Tuomisto, J.T., R. Pohjanvirta, M. Unkila and J. Tuomisto, 1999. TCDD-induced anorexia and wasting syndrome in rats: effects of diet-induced obesity and nutrition. Pharmacol Biochem Beha., 62: 735-42.

Winterbourne, C.C., R.E. Howkins, M. Brain and R.W. Carrell, 1975. The estimation of red cell SOD activity. J. Lab. Clin. Med., 85: 337-341.

Wright, R.S., J.W. Anderson and S.R. Bridges, 1990. Propionate inhibits hepatocyte lipid synthesis. Proc Soc Exp Biol Med., l95: 26-9.

Yalniz, M., I.H. Bahcecioglu, N. Kuzu, O.K. Poyrazoglu, O. Bulmus, S. Celebi, B. Ustundag, I.H. Ozercan, and R. Sahin, 2007. Preventive role of genistein in an experimental non alcoholic steatohepatitis model. J Gastroenterol Hepatol., 22: 2009-14.

Zhang, J., J. Liu, L. Li and W. Xia, 2008. Dietary chitosan improves hypercholesterolemia in rats fed high-fat diets Nutr Res., 28: 383-90.

Zhang, X., J. Yang, Y. Guo, H. Ye, C. Yu, C. Xu, L. Xu, S. Wu, W. Sun and H. Wei, 2010. Functional proteomic analysis of nonalcoholic fatty liver disease in rat models: enoyl-coenzyme a hydratase down-regulation exacerbates hepatic steatosis. Hepatology., 51: 1190-9.

Zhou, K., W.S. 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.