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

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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 aminotransferase (AST), and alkaline phosphatase (ALP) and decreased serum levels of total proteins, albumin and globulin. Moreover, high fat diet elevated hepatic malondialdehyde 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 anti-lipidemic, 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 steatohepatitis (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).
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>
<thead>
<tr>
<th>Table 1: Composition of experimental diets.</th>
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<tbody>
<tr>
<td>g/100 g diet</td>
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<tr>
<td>--------------</td>
</tr>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>Choline chloride</td>
</tr>
<tr>
<td>Vitamin mixture</td>
</tr>
<tr>
<td>Mineral mixture</td>
</tr>
<tr>
<td>Cholesterol</td>
</tr>
<tr>
<td>Corn oil</td>
</tr>
<tr>
<td>Beef tallow</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Chitosan</td>
</tr>
<tr>
<td>Wheat bran</td>
</tr>
<tr>
<td>Cellulose</td>
</tr>
<tr>
<td>Starch</td>
</tr>
</tbody>
</table>

*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 °C 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) \[\text{LDL-C} = \text{TC} - (\text{HDL-C} + \frac{\text{TAG}}{5})\].

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 H₂O₂ 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 mean that there is no significant difference between groups. The different letters mean 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Food intake (g/ rat/day)</th>
<th>Body weight gain (g/ 6 weeks)</th>
<th>Liver weight (g)</th>
<th>Relative weight of liver (g%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td></td>
<td>10.9±1.8 b</td>
<td>63.08±6.6 a</td>
<td>7.94±0.44 a</td>
<td>4.34±0.087 b</td>
</tr>
<tr>
<td>Chitosan</td>
<td></td>
<td>11.5±1.2 b</td>
<td>63.8±3.8 a</td>
<td>8.7±0.25 a</td>
<td>3.81±0.081 a</td>
</tr>
<tr>
<td>Wheat bran</td>
<td></td>
<td>10.8±1.05 b</td>
<td>60.66±6.3 a</td>
<td>8.1±0.42 a</td>
<td>3.7±0.108 a</td>
</tr>
<tr>
<td>High fat (Positive control)</td>
<td></td>
<td>8.6±1.02 a</td>
<td>91.2±5.3 c</td>
<td>11.14±0.46 c</td>
<td>5.5±0.22 c</td>
</tr>
<tr>
<td>High fat + Chitosan</td>
<td></td>
<td>9.1±0.98 ab</td>
<td>80.4±5.07 b</td>
<td>9.8±0.45 b</td>
<td>4.75±0.147 b</td>
</tr>
<tr>
<td>High fat + Wheat bran</td>
<td></td>
<td>9.14±0.79 ab</td>
<td>87.7±5.01 c</td>
<td>10.63±0.35 c</td>
<td>4.96±0.11 c</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative control</th>
<th>Chitosan</th>
<th>Wheat bran</th>
<th>High fat (Positive control)</th>
<th>High fat + chitosan</th>
<th>High fat + wheat bran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic MDA (nmol/g)</td>
<td>51.27±2.3a</td>
<td>49.6±1.55a</td>
<td>52.76±2.4a</td>
<td>55.06±1.7b</td>
<td>54.96±2.3a</td>
<td></td>
</tr>
<tr>
<td>Hepatic GSH (mg/g)</td>
<td>15.33±0.42a</td>
<td>15.08±0.39a</td>
<td>14.36±0.54a</td>
<td>15.2±0.48b</td>
<td>15.25±0.38a</td>
<td></td>
</tr>
<tr>
<td>Erythrocytes Cu-Zn SOD (U/ml)</td>
<td>299.17±5.38a</td>
<td>254.5±20.6a</td>
<td>285±16.4a</td>
<td>275.05±30.1b</td>
<td>231.08±29.6a</td>
<td></td>
</tr>
<tr>
<td>Plasma Catalase (U/ml)</td>
<td>82.9±1.13a</td>
<td>82.1±1.0a</td>
<td>79.0±2.52a</td>
<td>81.4±1.45b</td>
<td>80.3±0.72a</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative control</th>
<th>Chitosan</th>
<th>Wheat bran</th>
<th>High fat (Positive control)</th>
<th>High fat + chitosan</th>
<th>High fat + wheat bran</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>24.3±0.42a</td>
<td>23.4±1.6a</td>
<td>24.33±1.33a</td>
<td>28.6±1.08a</td>
<td>25.6±1.45a</td>
<td>26.4±0.63a</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>66.67±3.48a</td>
<td>65.67±2.06a</td>
<td>68.67±2.88a</td>
<td>62±2.29a</td>
<td>72.63±4.5a</td>
<td>81.4±1.16a</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>80.45±10.3a</td>
<td>83±7.02a</td>
<td>76.5±5.3</td>
<td>115±6.13a</td>
<td>96.03±4.2a</td>
<td>108±4.4a</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.34±0.02a</td>
<td>0.34±0.014a</td>
<td>0.34±0.138a</td>
<td>0.37±0.15a</td>
<td>0.35±0.014a</td>
<td>0.35±0.011a</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.16±0.03a</td>
<td>0.163±0.005a</td>
<td>0.162±0.01a</td>
<td>0.16±0.004a</td>
<td>0.17±0.004a</td>
<td>0.16±0.007a</td>
</tr>
<tr>
<td>Indirect bilirubin (mg/dl)</td>
<td>0.18±0.02a</td>
<td>0.177±0.015a</td>
<td>0.182±0.013a</td>
<td>0.181±0.013a</td>
<td>0.17±0.013a</td>
<td>0.19±0.009a</td>
</tr>
<tr>
<td>Total protein (mg/dl)</td>
<td>7.58±0.27a</td>
<td>7.51±0.27a</td>
<td>7.5±0.25a</td>
<td>6.4±0.31a</td>
<td>7.48±0.17</td>
<td>7.5±0.36</td>
</tr>
<tr>
<td>Albmin (mg/dl)</td>
<td>4.43±0.28a</td>
<td>4.05±0.23a</td>
<td>4.2±0.33a</td>
<td>2.95±0.25a</td>
<td>3.96±0.21a</td>
<td>4.5±0.145a</td>
</tr>
<tr>
<td>Globulin (mg/dl)</td>
<td>2.95±0.14a</td>
<td>3.49±0.23a</td>
<td>3.41±0.27a</td>
<td>3.5±0.42a</td>
<td>3.2±0.33</td>
<td>3.28±0.17a</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.5±0.13a</td>
<td>1.14±0.28a</td>
<td>1.29±0.18a</td>
<td>0.86±0.067a</td>
<td>1.32±0.165</td>
<td>1.57±0.075</td>
</tr>
</tbody>
</table>

Liver Function Parameters:

Serum AST, ALT and ALP activities were significantly (p < 0.05) elevated by 17.6%, 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-inflammatory 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


