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Ameliorating effects of chitosan and wheat germ on hyperlipidemia in experimental rats

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Hyperlipidemia is a predominant risk factor that contributes to the advancement and development of atherosclerosis and resulting cardiovascular disease, which is one of the most genuine diseases in human. The present study aims to assess the hypolipidemic impacts of Chitosan and wheat germ. Male (Sprague-Dawley) were divided into six groups: group 1 was normal rats (negative control) fed on basal diet, whereas groups (2, 3, 4, 5 and 6) were hyperlipidemic rats. Group 2 (positive control) was fed on high fat diet , group 3 fed on basal diet and groups 4, 5 , 6 were fed on basal diet and receiving 5% chitosan (CH), 10 % wheat germ (WG) and 7.5 % mixture of them respectively. The nutrition period continued for 6 weeks. At the end of the experimental period, fasting blood samples were obtained from rats in all groups and analyzed for several biochemical parameters. The results obtained showed that treated groups (3, 4, 5 and 6) caused a significant decrease in body weight as compared to the positive control group (2). Serum total lipids, total cholesterol, LDL cholesterol and triglycerides, glucose and malondialdehyde were decreased in treated groups fed with Chitosan, wheat germ and their mixture diet compared to the positive control group (2) and basal diet treated group (3).The total antioxidant capacity, liver and kidney functions, , and HDL cholesterol were ameliorated. Histopathological examination showed that positive control rats fibroplasia in portal triad with appearance of newly formed bile ductuoles, cytoplasmic vacuolization of hepatocytes and lipidosis of hepatocytes compared with negative control and treated groups. The attained results demonstrated that Chitosan and the mixture of Chitosan and wheat germ supplementation can protect against the progress of hyperlipidemia.

Keywords: Chitosan, Hyperlipidemia, Lipid profile, Liver function, Total antioxidant capacity, Wheat germ.

INTRODUCTION

Numerous reports have concentrated on the most proficient method to reduce plasma lipid concentrations and the absorption of fat in the intestinal tract to decrease chronic diseases associated with diet. Dietary fiber, for example, Chitosan, pectin and psyllium demonstrate some potential hypolipidemic impact (Zhang *et al*., 2008). Chitosan is a derivative of chitin, natural polymer of Glucosamine and N-acetyl Glucosamine derived from the shells of crustaceans such as shrimps, crabs and lobsters (Woo, 2003). It is the most plentiful natural polymer after cellulose (Muzzarelli, 2000) .Various investigations have found that Chitosan has a gainful decreasing impact on plasma cholesterol, which may assume a vital role in the counteractive action and treatment of cardiovascular diseases (Razdan and Pettersson, 1996; Miura *et a*l., 1995). It seems to bind to negatively charged lipids in animal trials, subsequently diminishing their gastrointestinal uptake (Deuchi *et al*., 1994) and bringing down serum cholesterol (Ormrod *et al*., 1998). Some human trials have recommended

that chitosan may reduce body weight (Schiller et al., 2001). Ernst and Pittler proposed a more prominent weight reduction with Chitosan contrasted placebo. Because of its biodegradable, biocompatible and nontoxic nature, remarkable consideration have been centered on Chitosan to develop Nutraceuticals (Muzzarelli, 1994). In light of ongoing discoveries, Chitosan display a diversity of natural biological activities including hypolipidemic, antidiabetic, hypocholesterolemia, immune-stimulating, antioxidant, anti-inflammatory and antimicrobial activities that provide them the ability to be highly effective Nutraceuticals (Wenshui *et al*., 2010). The wheat germ is a by-product of the flour processing industry and is the most vital and magnificent sources of proteins, fibers, vitamins, and minerals at a relatively minimal cost (Nichelatti and Hidvegi, 2002). Wheat germ is the most critical nutritious part of the wheat germ grain isolated by ultra-modern milling technology, which holds free radical that turn helps to inhibit diabetes, heart diseases and cancers. A striking advantage of wheat germ lipids was the generally high content state of polyunsaturated fatty acids (PUFA), particularly linoleic fatty acids , the essential fatty acid, is one of the most essential polyunsaturated fatty acids in human nourishment in light of its counteractive action of cardiovascular coronary diseases (CHD).With respect to the issue of fatty acids, the linoleic fatty acids (belonging to the ω-6) and α-linolenic fatty acids (belonging to the ω-3) are considered essential, as they must be obtained from food and cannot be synthesized by mammals(Mahmoud *et al*.,2015). Several studies have demonstrated that ω-3fatty acids have benefits for decreasing CHD hazard. It has been also proposed that ω-3/ ω-6 ratio of 10 or less results in a decrease in a lethal CHD hazard (Dunford and Zhang, 2003).Therefore, the aim of the present study was to investigate the effects of Chitosan and wheat germ on serum lipids in hyperlipidemic rats.

MATERIALS AND METHODS

Chemicals and reagents Ingredients:

The ingredients used for the preparation of the diet given to the animals were purchased from the local market. These items were corn starch, and corn oil. Casein was attained from Sisco Research Laboratories PVT.LTD., India. Salts and vitamins used for the preparation of the salt and vitamin mixtures were obtained from Merck, Germany and composed as indicated by (AIN 95) according to (Reeves *et al*.,1993)

Wheat germ: was acquired from south Cairo and Giza flour Mills and Bakeries Company, Giza, Egypt.

Reagents: All biochemical parameters were purchased from Bio-diagnostic Company, Giza, Egypt.

Preparation of Chitosan: Isolation of chitin and preparation of Chitosan was prepared according to the method described by (Alsagheer *et al*, 2009)

Experimental animals

Forty-two Sprague-Dawley male albino rats weighing 110 ±10 g were obtained from the Laboratory Animal House, National Research Centre, Giza, Egypt.

Design of animal experiment

The experiment consists of two stages:

The first stage was carried out to induce a state of hyperlipidemia. This induction was conducted by feeding thirty-five rats for 6 weeks with high fat diet (HFD) which was prepared as basal diet except that 10% corn oil portion was substituted with 10% sheep fat and it was supplemented with 1% cholesterol and 0.25% bile salts (Fukushima,1997).

At the end of the first stage, fasting blood samples were obtained to estimate serum glucose level, total lipid, total cholesterol, triglycerides HDL-cholesterol and LDL-cholesterol.

The second stage was carried out by dividing the hyperlipidemic rats into five groups (7 rats each). Another group of 7 normal rats was fed on the basal diet consisting of corn starch 65%, casein 15%, corn oil 10%, salt mixture 4%, vitamins mixture 1% and cellulose 5% (AOAC, 2000) and were considered as negative control. The experimental groups were classified as follows:

Group 1: negative control (normal rats)

Group 2: hyperlipidemic rats were fed on high fat diet till the end of the experiment (positive control)

Group 3: hyperlipidemic rats were fed on basal diet (basal diet treated group)

Group 4: hyperlipidemic rats were fed on basal diet + 5% chitosan

Group 5: hyperlipidemic rats were fed on basal diet + 10% wheat germ

Group 6: hyperlipidemic rats were fed on basal diet + 7.5 mixtures of Chitosan and wheat germ.

All animals were kept individually in stainless steel cages and water was allowed *ad- libitum.* This stage was extended for 6 weeks. During this experimental period, food consumption and body weight of the animals were determined.
The experimental procedure was carried out according to the institutional Animals Ethics Community of the National Research Centre, Egypt.

Blood sampling

At the end of the experiment (6 weeks), the rats were fasted overnight. All the animals were scarified by cervical decapitation. Blood samples were collected from each rat from the retro-orbital vein and were received into clean dry centrifuge tubes. Serum was separated by centrifugation at 3000 RPM for 15 minutes and kept in deep-freezer at 20˚C until analysis.

Nutritional and Biochemical analysis

The nutritional assessment included food consumption, gain in body weight and feed efficiency ratio calculated as a result of dividing the gain in body weight by food consumption. The biochemical analysis was done by the following parameters**:** Serum glucose was determined according to (Trinder, 1969). Serum total lipids (TL), triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were determined according to (Knight *et al.,* 1972., Fossati and Prencipe, 1982; Allain *et al*., 1974; Levy, 1981; Burstein *et al*., 1970) respectively.
Atherogenic Index was calculated according to Lee and Niemann (1996) using the following equation.

$$\text{Atherogenic Index (AI)} = \frac{\text{Total Cholesterol-HDL-C}}{\text{HDL-C}}$$

Concerning Liver function biomarkers, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured calorimetrically in serum according to the method described by Reitman and Frankel (1957). Alkaline phosphatase (ALP) activity in serum was determined according to (Rec, 1972). Urea level was determined according to the method described by (Patton and Crouch, 1977) and creatinine level was determined according to the method described by Faulkner and King (1976). On the other hand, total antioxidant capacity was determined according to the method described by Koracevic *et al*, (2001) and malondialdehyde was determined according to the method described by (Satoh, 1978).

Histopathological examination

Liver of the sacrificed rats was taken and immersed in 10 % formalin solution. The specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Dehydrated specimens were cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with heamtoxylin and eosin for histopathological examination according to the method described by (Carleton, 1980).

Statistical analysis

Data are presented as mean ± SE. Statistical analysis of the data was performed using (SPSS, 1999). Unpaired student's t-test was used to compare biological differences. Meanwhile, one-way analysis of variance (ANOVA) was used for comparison of different biochemical values in various experimental groups. It was followed by Duncan's multiple range test to clarify the significance. P values are less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Table 1: Body weight gain, food intake and feed efficiency ratio of the different experimental groups.

Parameters / Treatments	Body weight gain (BWG) (g)	Food intake (FI) (g)	Feed efficiency ratio (FER)
G1	80.5 ± 2.23^{b}	549.5 ± 8.49^{c}	0.14 ± 0.003^{a}
G2	90 ± 1.95^{a}	613.57 ± 3.96^{a}	0.14 ± 0.003^{a}
G3	89.16 ± 2.25^{a}	592.16 ± 4.25^{b}	0.15 ± 0.004^{a}
G4	70.14 ± 2.53^{c}	545.71 ± 8.21^{c}	0.12 ± 0.005^{b}
G5	69.28 ± 2.08^{c}	556.85 ± 8.95^{c}	0.12 ± 0.004^{b}
G6	68.42 ± 2.23^{c}	547.71 ± 7.85^{c}	0.12 ± 0.004^{b}

All values are means ± S.E. Means with different letters are significantly different at p<0.05.Negative control (G1), positive control (G2), basal diet treated (G3), Chitosan 5% (G4), Wheat germ 10% (G5) mixture of chitosan and wheat germ 7.5% (G6).

The Body weight gain, food intake and feed efficiency ratio of the diverse trail groups are appearing on the table (1). Results showed that positive control caused a significant increase (90 ± 1.95g) in body weight gain in comparison with the normal negative control (80.50±2.23g). Supplementation of Chitosan (5%), wheat germ (10%) and their mixture (7.5%) as treated groups (4,5and6) caused a significant decrease in body weight gain as compared to positive control and basal diet treated group(70.14±2.53, 69.28± 2.08, 68.42±2.23, 90 ± 1.95 and 89.16±2.25g respectively). No significant difference was found in the treated group (4, 5 and 6). Furthermore, the food intake results revealed that the positive control group showed a significant increase (613.57 ± 3.96g) in food intake as compared to negative control (549.50 ± 8.49g). There were significant differences in the food intake of the Chitosan, wheat germ or their mixture groups (545.71± 8.21, 556.85± 8.95 and 547.71± 7.85 g) as compared with basal diet treated group (592.16± 4.25g) and positive control groups (613.57 ± 3.96). Also the feed efficiency ratio (FER) presented in the same table, showed that Chitosan 5% group recorded the same value of the wheat germ and their mixture (0.12). Although the data showed no significant changes among the supplemented group (4, 5 and6) but there were significant changes compared with both negative and positive control were observed. The results are in accordance with those of (Jayasooriya *et al*., 2000; Barakat and Mahmoud, 2011) who stated that rats fed with high cholesterol diet indicated a significant increase in body weight and liver weight, which leads to secondary complications clinically. In this study, the relative organ weight in hyperlipidemic rats was decreased significantly following treatment with Chitosan, wheat germ and their mixture. The hypolipidemic and antiatherogenic effects of chitosan and wheat germ may be responsible for the beneficial action of their fibers on body weight gain and liver weights. In this study, the relative organ weight in hyperlipidemic rats was diminished significantly following treatment with Chitosan, wheat germ and their mixture. The hypolipidemic and antiatherogenic impacts of Chitosan and wheat germ may be attributed to the potential activity of their fibers on body weight gain and liver weights. The present data concurred with previous investigations announced that rats fed a semi purified diet supplemented with 5% Chitosan for 31 days indicated decrease in food intake and body weight gain contrasted with control(Kondo and Osada,1996) .This study showed that both Chitosan and wheat germ or their mixture significantly enhanced BWG and FER of the hyperlipidemic groups when contrasted with positive control group and in accordance to the results by(Gallaher et al., 2000) who reported that the liver and body weights of the rats fed on Chitosan were significantly lower than those nourished on control diet. The reduced level of growth of chitosan group was due to a decrease food intake. Also, the lowering of body weight gain in rats was clear for chitosan treatment. This may show that chitosan could be utilized as a weight loss agent for both obese and healthy human because of its binding of lipids in the gastrointestinal tract to decrease fat absorption (Shields *et a*l., 2003). Another examinations was carried out human demonstrated that Chitosan was potent in stimulating weight reduction and decreasing body fat in obese adults (Zhang et al., 2008; Schiller et al., 2001;Shields et al., 2003).

Data in Table (2) illustrated that serum glucose level of positive control was significantly higher than that of normal controls (134.34 ± 7.52, 83.5 ± 2.32 mg/dl, respectively). On the other hand, significant decrease in serum glucose level was observed in all treated groups 4, 5, 6 (81.14 ± 2.84, 86.08 ± 2.54 and 87.42±2.80 mg/dl, respectively) compared with basal diet treated group (113.93±7.05mg/dl) and positive control groups (134.34 ± 7.52). It was noticed that the effect of Chitosan supplement (5%) is better than the effect of both wheat germ and their mixture on glucose level of hyperlipidemic rats. No significant difference in serum glucose between negative control and treated groups was observed.

These findings are in agreement with those acquired by (McIntosh and Miller, 2001) who reported that diets containing high levels of dietary fibers has been appeared to enhance glycemic control and diminish hyperlipidemia in diabetic patients.

Moreover, these data are compatible with Yao et al., 2008) who suggested the potential of high molecular weight chitosan in decreasing glucose in Streptozotocin-induce diabetic rats. These results are in concurrence with (Cara *et al*., 2001) who stated that wheat germ is a good source of pyhtosterol, and one such phytosterol is beta sistosterol manages glucose and insulin levels in type 2 diabetes by enhancing the release of insulin concentrations and suppressing glucose-6-phosphatase.

Table 2: Levels of glucose (mg/dl), total antioxidant capacity (mM/L) and malondialdehyde (nmol/ml) of the different experimental groups.

Parameters / Treatments	Glucose (mg/dl)	Total antioxidant capacity (m M/L)	Malondialdehyde (nmol/ml)
G1	83.5 ± 2.32^{c}	1.56 ± 0.01^{b}	3.58 ± 0.18^{c}
G2	134.34 ± 7.52^{a}	1.38 ± 0.01^{d}	5.18 ± 0.23^{a}
G3	113.93 ± 7.05^{b}	1.44 ± 0.02^{c}	4.5 ± 0.16^{b}
G4	81.14 ± 2.84^{c}	1.64 ± 0.02^{a}	2.61 ± 0.076^{d}
G5	86.08 ± 2.54^{c}	1.59 ± 0.02^{ab}	2.81 ± 0.21^{d}
G6	87.42 ± 2.80^{c}	1.66 ± 0.01^{a}	2.51 ± 0.14^{d}

All values are means ± S.E. Means with different letters are significantly different at p<0.05
Negative control (G1), positive control (G2), basal diet treated (G3), Chitosan 5% (G4),
Wheat germ 10% (G5), mixture of chitosan and wheat germ 7.5% (G6).

Serum total antioxidant capacity (TAC) level of (positive control) was significantly reduced (1.38± 0.01mM/L) as compared to normal control (1.56± 0.01 mM/L). However, a significant increase in the serum TAC level was observed in all treated groups 4, 5, 6 (1.64 ± 0.02, 1.59 ± 0.02 and 1.66 ± 0.01 mM/L, respectively) compared with basal diet treated group (1.44 ± 0.02 mM/L) and positive control (1.38± 0.01 mM/L). It was noticed that the mixture of Chitosan and wheat germ (7.5%) supplement had a better effect on TAC compared to either Chitosan alone or wheat germ. These data concur with Anraku *et al*. (2009) who reported that Chitosan may decrease certain levels of pro-oxidants, for example, cholesterol and uremic toxins in the gastrointestinal tract, thereby suppressing the subsequent progress of oxidative stress in the systemic circulation. The antioxidant properties of wheat grain might be related to the antioxidant components which present in the bran like phenolic acids, phytoestrogens and lignans (Slavin, 2004). Supplementation with antioxidant inhibit many diseases related to a high-fat (Xu *et al*., 2009; Hong *et al*., 2009). In addition, it has been shown that antioxidant supplements can successfully decrease the atherogenic lipoprotein profile in patients with hyperlipidemia and atherosclerosis (Diaz *et al*., 1997). Another conceivable impact of plant sterols, is their antioxidant activity as revealed by Wang, (2002) who reported that antioxidant activity action might be in part attributed to sterol content.

Malondialdehyde is the most critical marker of lipid peroxidation, because of the oxidative decomposition of specific macromolecules for example, lipids (Hazarika *et al*., 2003; Kavitha and Rao, 2007). Since, membrane lipid is highly capable of peroxidation, the free radicals effectively peroxidized the membrane lipids, and therefore MDA was formed (Sánchez-Moreno *et a*l., 2003). It was suggested that an increase in MDA levels of both kidney and liver tissues improved lipid peroxidation, which lead to tissue injury and failure of antioxidant, scavenging mechanism (Gholami-Seyedkolaei *et al*., 2013; Sloss, 2009). In concerning with MDA level increased significantly in the positive control group (5.18 ± 0. 23nmol/ml) as compared to negative control (3.58 ± 0.18 nmol/ml). The addition of Chitosan, wheat germ and their mixture showed significantly decreased MDA level in all treated groups (2.61 ± 0.07, 2.81 ± 0.21 and 2.51± 0.145nmol/ml, respectively) as compared to the positive control group (5.18 ± 0.23nmol/ml) and basal diet treated group (4.5 ± 0.16nmol/ml). No differences were found among the treated groups 4, 5 and 6. These results are in agreement with (She *et al*.,2013) who reported that Chitosan treatment significantly lowered the plasma MDA concentration when compared with untreated high fat fed animals and furthermore revealed that supplementation of Chitosan reduced oxidative stress in rats when contrasted with the control. These data agreed with (Liu *et al*.,2009) who stated that Chitosan oligosaccharides have preventive effects on suppressing the production of lipid peroxidation, like, malondialdehyde, restoring activities of endogenous antioxidants including superoxide dismutase and glutathione. However, administration of Chitosan prevented the build-up of oxidative stress by restoring normal antioxidant activity.

Table 3: Serum total lipid, total cholesterol, triglycerides, LDL-C, HDL-C (mg/dl) of the different experimental groups.

Parameters / Treatments	Total lipid (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	AI
G1	332.33 ± 6.76^{c}	120.16 ± 1.57^{c}	110.5 ± 1.74^{c}	38.5 ± 1.76^{c}	43.33 ± 5.85^{c}	1.77
G2	1107.45 ± 56.2^{a}	235.57 ± 3.92^{a}	321.57 ± 25.0^{a}	192.4 ±12.00^{a}	33.57 ± 2.26^{d}	5.86
G3	678.83 ± 80.91^{b}	198.50 ± 1.94^{b}	210.66 ± 25.3^{b}	136.66 ± 2.64^{b}	39 ± 3.91^{c}	4.08
G4	439.42 ±11.57^{c}	106.57 ± 1.78^{c}	91.7 ± 9.56^{c}	41.28 ± 1.68^{c}	61.71 ± 3.09^{a}	0.72
G5	399.71 ± 11.87^{c}	107.14 ± 1.51^{c}	91 ± 8.22^{c}	46.14 ± 2.08^{c}	55.21 ± 3.94^{b}	0.94
G6	355.28 ± 21.30^{c}	105.40 ± 2.67^{c}	98.42 ± 6.67^{c}	43.42 ± 1.52^{c}	57.71 ±1.78^{b}	0 .82

All values are means ± S.E. Means with different letters are significantly different at p<0.05. AI**;** Atherogenic Index**.** Negative control (G1), positive control (G2), basal diet treated (G3), Chitosan 5% (G4), Wheat germ 10% (G5), mixture of chitosan and wheat germ 7.5% (G6).

Table4: Serum urea and creatinine (mg/dl) of the different experimental groups.

Parameters / Treatments	Urea (mg/dl)	Creatinine (mg/dl)
G1	19.33 ± 0.73^{c}	0.77 ± 0.09^{cd}
G2	47.07 ± 2.58^{a}	2.19 ± 0.09^{a}
G3	35.35 ± 1.40^{b}	1.4 ± 011^{b}
G4	21.03 ± 1.85^{c}	0.62 ± 0.04^{d}
G5	23.51 ± 0.46^{c}	0.87 ± 0.08^{c}
G6	19.91 ± 1.21^{c}	0.65 ± 0.04^{cd}

All values are means± S.E. Means with different letters are significantly different at p<0.05. Negative control (G1), positive control (G2), basal diet treated (G3), Chitosan 5% (G4), Wheat germ 10% (G5), mixture of chitosan and wheat germ 7.5% (G6).

Tocopherol is represented as one of the plentifully structural components of wheat germ. Due to its free radical scavenging activity, it is considered as a strong antioxidant as reported by (Lin et al., 2004) who exhibited that wheat germ oil contains compounds, such as carotenoids and phytosterols which may participate in antioxidant activity. Moreover, it is possible that the phenolic compounds found in wheat germ oil also have free radical scavenging (Niu et al., 2011; Zhu et al., 2011).

Data in Table (3) illustrated that rats fed on hyperlipidemia-induced diet (positive control) showed significant increase in serum total lipids (1107.45 ± 56.26mg/dl), total triglycerides (321.57 ± 25.08mg/dl), total cholesterol (235.57 ± 3.92mg/dl), low density lipoprotein cholesterol (192.4 ± 12.00mg/dl) and atherogenic Index (AI) (5.86) compared with normal control group (332.33 ± 6.76, 110.5 ± 1.74, 120.16 ± 1.57, 38.5 ± 1.76 and 1.77 mg/dl respectively). Supplementation with Chitosan (5%), wheat germ (10%) and their mixture (7.5%) showed significant decrease in total lipids, triglycerides, total cholesterol, LDL-C and AI and significant increase in HDL-C compared with hyperlipidemic group (positive control) as shown in Table (3). The current study is in agreement with results suggested by (Makni et al., 2008) who demonstrated that the elevation in HDL-C or HTR ratio (HDL-C/TC) is the most essential criteria of anti-hypercholesterolemic agent. (Zhang et al., 2008) found the hypolipidemic action of Chitosan functions both in healthy and hypercholesterolemic rats. Several researchers demonstrated that Chitosan can decrease plasma and liver TG and TC levels showing hypocholesterolemic and hypolipidemic impacts (Maezaki et al., 1993; Cho et al., 1998). It has been accounted for that Chitosan has powerful fat-binding activity in vitro (Zhou *et al*., 2006). Furthermore, Chitosan was also shown to elevate fecal-neutral-steroid and bile-acid excretion in rats 37, 60 and decrease the postprandial plasma triglyceride level in broiler chickens (Razdan and Pettersson, 1994). Ormrod et al., 1998; Liu et al., 2008a) indicated that rats fed hypercholesterolemia-induced diets containing chitosan significantly decrease plasma cholesterol and LDL-C. The decrease of fatty acid and bile

acid will cause less absorption of fat from the diets (Gallaher et al., 2000) and decrease the endogenetic cholesterol due to the disturbance of enterohepatic bile acid circulation (Miura et al., 1995).Besides, the present findings concur with previous study set up that the supplementation of 7% wheat germ with a high-fat and elevated cholesterol diet enhanced lipoprotein in rats (Lairon et al., 1987). Soluble protein components of wheat germ are known to suppress pancreatic lipase activity (Lairon et al., 1985). In rats, the absorption of cholesterol and triglycerides was delayed and decreased by wheat germ and other wheat fractions in part as a result of the inhibition of pancreatic lipase and the reduction in triacylglycerol lipolysis (Borel et al., 1989). Cara et al., (1991) reported that wheat germ plays an effective role in the dietary administration of hyperlipidemic subjects. Also, he demonstrated that serum triglycerides response was significantly lower in the presence of wheat germ in rats fed cholesterol-diet. In addition to Cara et al., (1992) suggested that addition of wheat germ in a meal decrease plasma chylomicron cholesterol concentrations by 27.1% more than a few hours in subjects.

Data in Table (4) shows the results of the kidney functions. Urea level records an increase in positive control (47.07 ± 2.58mg/dl) as compared to negative control (19.33 ± 0.73mg/dl). The addition of Chitosan, wheat germ and their mixture to the diets of treated groups (4, 5 and 6) significantly decreased the level of serum urea (21.03 ± 1.85, 23.51 ± 0.46 and19. 91 ± 1.21mg/dl, respectively) as compared to the positive control and the basal diet treated group (47.07 ± 2.58 and 35.35 ± 1.40 mg/dl respectively). However, no change among the treated groups and the negative control was evident. The best result was recorded in the mixture of Chitosan and wheat germ (19.91 ± 1.21mg/dl).

With regard to the creatinine concentration, there was significant increase in positive control (2.19± 0.09mg/dl) as compared to negative control (0.77±0.09mg/dl).Supplementation of Chitosan, wheat germ and their mixture to the treated groups (4, 5 and 6) significantly decreased the level of serum creatinine (0.62 ± 0.04, 0.87 ± 0.08 and 0.65 ± 0.04mg/dl respectively) as compared to basal diet treated and positive control group (1.4 ± 011 and 2.19 ± 0.09mg/dl respectively). No difference in creatinine concentration was recorded when compared to the negative control group. This could be related to the fact that consumption of food rich in dietary fibers motivates the extra renal route of nitrogen excretion. Younes et al.,(1998) found that indigestible carbohydrate/dietary fibers increased cecal blood flow and fecal weight , leading to accelerated diffusion of blood urea into the cecal lumen (by three fold), urea lysis to ammonia, protein synthesis by the microflora, and increased fecal excretion of nitrogen. Thus, decrease the role of kidney in the excretion of nitrogen and lower blood urea concentration. Barakat *et al*. (2011) revealed that addition of wheat germ to the diet of nephrotoxic rats caused significant ameliorating in kidney function.

Table (5) showed the results of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities (IU/L**)** of the hyperlipidemic studied groups. Data showed that there were significant increases in the activities of AST and ALT in positive control (75.00 ± 8.32, 82.71 ± 8.44 IU/L, respectively) as compared to negative control (33.25 ± 9.38, 29.5 ± 3.04 IU/L respectively). The addition of Chitosan, wheat germ and their mixture showed a significant decrease in all treated groups (4,5 and 6) compared to the basal diet treated group (59.83 ± 10.21, 50.00 ± 5.72 IU/L, respectively) and positive control (75.00 ± 8.32, 82.71 ± 8.44 IU/L respectively). There were no significant differences between treated groups (4, 5, and 6) and negative control group.

ALP activity showed a significant increase in positive control (150.50 ± 17.27 IU/L) as compared to negative control (68.27 ± 6.45 IU/L). The addition of Chitosan, wheat germ and their mixture significantly reduced ALP activity in all treated groups (67.42 ± 8.67, 60.89 ± 3.39, 59.5 ± 5.69 IU/L respectively) as compared to positive control and basal diet treated group. The best effect for liver functions was obtained by feeding a mixture of wheat germ and chitosan (7.5%) to treated groups.

Morphological changes that happen in the liver influence several metabolic processes in the organism. Peroxide formation induced by hypercholesterolemia result in the release of some enzymes by interacting with cellular structure and function (Sudhahar et al., 2007). Thus, the enzyme activity such as alkaline phosphatase, lactate dehydrogenase, and transaminases increase.

HISTOPATHOLOGICAL EXAMINATION

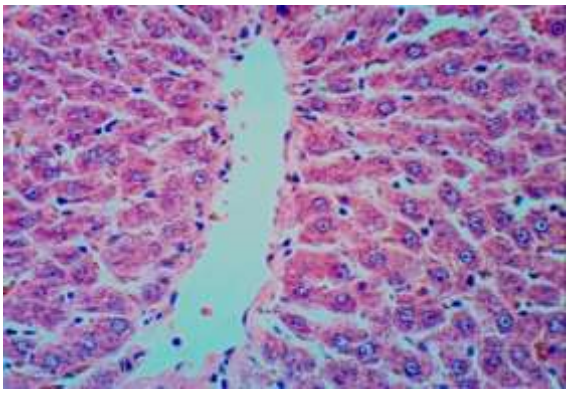

Fig.1.liver of rat group 1 showing the normal histological structure of hepatic lobule (H and E x400)

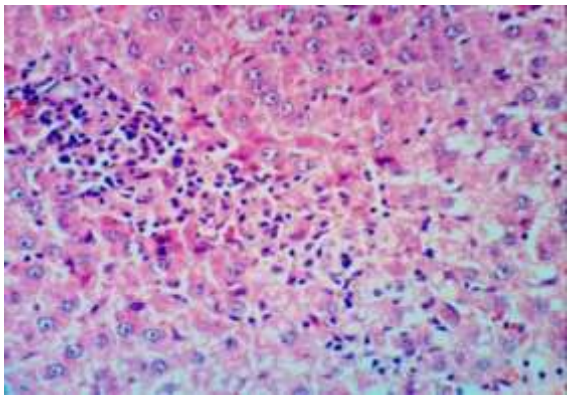

Fig.2.liver of rat from group 2 showing fibroplasia in portal triad with appearance of newly formed bile ductuoles, cytoplasmic vacuolization of hepatocytes (H and E x400)

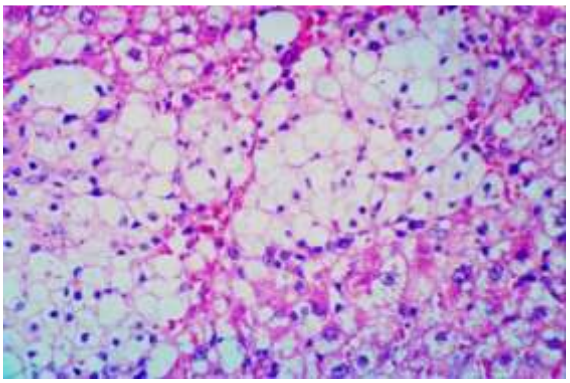

Fig.3.liver of rat from group 3 showing lipidosis of hepatocytes and congestion of hepatic sinusoids (H and E x400)

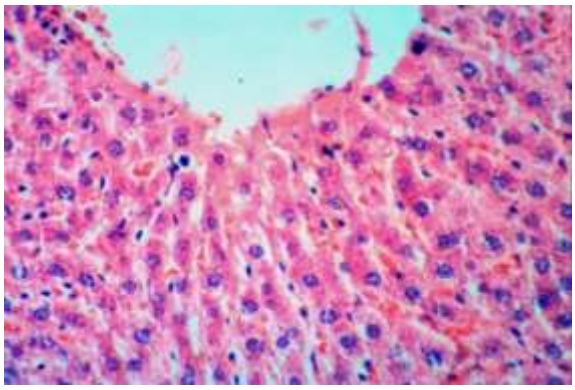

Fig.4.Liver of rat from group 4 showing kupffer cells activation and slight congestion of hepatic sinusoids (H and E x400)

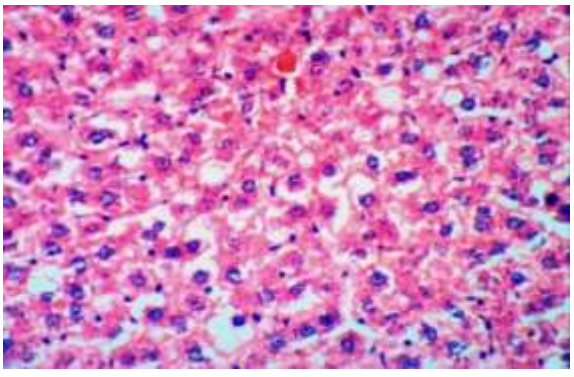

Fig.5.Liver of rat from group 5 showing slight vacuolation of hepatocytes (H and E x400)

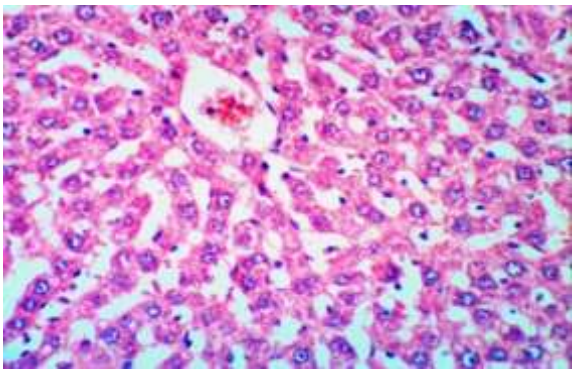

Fig.6.liver of rat from group 6 showing slight dilatation of hepatic sinusoids and kupffer cells activation (H and E x400)

Table 5: Serum ALT, AST and ALP activities (IU/L) in the Different experimental groups.

Parameters / Treatments	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
G1	33.25 ± 9.38^{c}	29.5 ± 3.04^{c}	68.27 ± 6.45^{c}
G2	75.00 ± 8.32^{a}	82.71 ± 8.44^{a}	150.50 ± 17.27^{a}
G3	59.83 ±10.21^{b}	50.00 ± 5.72^{b}	88.55 ± 3.61^{b}
G4	37.42 ± 8.80^{c}	37.4 ± 3.03^{bc}	67.42 ± 8.67^{c}
G5	33.57 ± 7.92^{c}	29.85 ± 2.16^{c}	60.89 ± 3.39^{cd}
G6	25.92 ± 8.78^{c}	29 ± 1.86^{c}	59.50 ± 5.69^{d}

All values are means ± S.E. Means with different letters are significantly different at $p<0.05$.
Negative control (G1), positive control (G2), basal diet treated (G3), Chitosan 5% (G4), Wheat germ 10% (G5), mixture of chitosan and wheat germ 7.5% (G6).

With the increase in cellular membrane permeability, intracellular fluid transfers onto intracellular space, resulting in muscle and liver cell degeneration. The increased activities of aminotransferases and alkaline phosphatase are the result of leakage from damaged cells, and are used as indicators of liver damage, especially for non-alcohol fatty liver diseases (NAFLD) (Giannini et al., 2005). Feeding with Chitosan decreased the abnormal aminotransferase activity in rats fed the high fat diet. The present study is in agreement with results obtained by Ahmed et al., (1987) who found that hypercholesterolemia state significantly stimulated AST and ALT activity in the plasma. ALT activity slightly lowered in rats fed hypercholesterolemia-induced diets containing different levels of chitosan compared to hypercholesterolemic control. Osman *et al*, (2010) found that hypercholesterolemia causes were released of enzymes such as ALT, AST and LDH into the blood. Increasing in enzyme activity was directly proportional to the degree of cellular damage. Supplementation with Chitosan lowered the level of previous enzymes. These results are also in accordance with (Ahmed et al., 2014) who found that Chitosan and the mixture of Chitosan and wheat germ caused a significant decrease in the level of enzymes such ALT, AST and ALP in diabetic rats. Maha, (2011) revealed that the activity of Chitosan and wheat bran can protect against high fat diet-induced hepatic steatosis was evident by significantly increasing in all liver parameters with regard to the control and treated groups.

CONCLUSION

The present study indicated that Chitosan, wheat germ and their mixture decrease hyperlipidemia through enhancing lipid profiles, liver and kidney functions, blood glucose level, decreasing oxidative stress and ameliorate histological alteration.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

SIGNIFICANCE STATEMENTS

The aim of the present study was to investigate the effects of Chitosan and wheat germ on serum lipids in hyperlipidemic rats. The results of this study recommended that chitosan and the mixture of Chitosan and wheat germ have potential handiness as a natural supplement or functional food for treating and preventing hyperlipidemia.

AUTHOR CONTRIBUTIONS

Ahemd FA, proposed the idea of research and performed isolation and extraction of compounds. Also, he analyzed all the data and revised manuscript. Abdel-Lattife MS designed and performed the experiments and also wrote the manuscript. Abdel- Azeem AS, Hegazy AM, Hassouna HZ and Algalaly MA performed animal treatments experiments, blood sampling, tissue collection, nutritional and biochemical analysis, data analysis and reviewed the manuscript. All authors read and approved the final version.

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