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Effect of Chitosan (From the Exoskeletons of Shrimp) on Body Weight and Blood Lipid Profile in Rats

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Abstract: Chitosan is a dietary fiber which obtained as a by-product from shellfish processing. It is a polysaccharide deacetylated from Chitin, that constitutes the exoskeletons of shrimp and other crustaceans.The present study was carried out to investigate the effect of Chitosan alone and mixture of Chitosan with certain vitamins (Vitamin C and Folic acid) on Body weight, serum lipid profile and liver histology in rats. Twenty four Sprague-Dawley rats divided into 4 groups and fed on basal diet (control group), diet supplemented with 2% Chitosan, Chitosan+1g Vitamin C /kg diet and Chitosan+40mg Folic acid / kg diet, for 4 weeks.The studied parameters included: food intake, body weight,% liver weigt/ body weight, FER, Total cholesterol, Triglycerides, HDL-C, LDL-C,VLDL-C, and the histological changes in rats liver. Results showed that, there was a highly significant ($P \leq 0.01$) decrease of body weight, Triglycerides, VLDL-C in group fed on Chitosan+vitamin C. In contrast, there was a significant ($P \leq 0.01$) increase of Triglycerides and VLDL-C in group fed on Chitosan 2%, when compared with control group. Histological changes were observed in rats group fed on Chitosan mixed with Vitamin C. Our findings provide evidence that; the supplementation of mixtures of Chitosan with certain vitamins might be of beneficial effect on obesity and the incidence of hyperlipidemia.

Key words: Chitosan · Vitamin C · Folic acid · Body weight · Triglycerides · VLDL-C

INTRODUCTION

Hypercholesterolemia is mostly due to a combination of environmental and genetic factors, that include obesity and dietary choices [1]. Elevated blood cholesterol level is due to abnormalities in the levels of lipoproteins that carry cholesterol in the bloodstream. This may be related to diet, as daily intake of an adequate amount of dietary fiber is the most common recommendation for the prevention and treatment of constipation and has an important role in the maintenance of normal bowel function. Results from various studies have demonstrated that adequate fiber intake has many health benefits and may prevent or decrease an individual's risk of developing coronary disease [2]. Hypercholesterolemias promote ischemic tissue damage by enhancing the vulnerability of the microcirculation to the deleterious effects of ischemia and other inflammatory stimuli [3]. This leads to an increase in the incidence of myocardial ischemia and cardiac events [4]. Chitosan (CS), a polysaccharide deacetylated from chitin, is a hardsubstance that constitutes the exoskeletons of shrimp, lobster, crabs and other crustaceans. Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) [5].

It was found that Chitosan is the only abundant polysaccharide derived from animals and its cationic characteristics are different from other dietary fibers [6]. Liu *et al.* [7] stated that, Chitosan is a natural and nontoxic polysaccharide and it has exhibited a potent hypocholesterolemic activity in rats. Moreover, it exhibits a marked hypolipidemic activity that would reduce the risk of cardiovascular diseases [8]. In recent years, many reports have focused on how to decrease plasma lipid concentrations and absorption of fat in the intestinal tract to reduce diet-related chronic disease. The fibrous property of chitosan (CS) has attracted the attention for its use in a wide variety of application; Chitosan is increasingly being recognized as a weight– reducing agent for the obese and as effective materials in the management of hypercholesterolaemia [9, 10]. Chitosan is positively charged and soluble in acidic to neutral solution with a charge density dependent on pH and

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the % Degree of Acetylation value (DA). This makes chitosan a bioadhesive, which readily binds to negatively charged surfaces such as mucosal membranes [11] Chitosan supplements are marketed as fat blockers or trappers, it blocks the absorption of as much as 120 g of dietary fat per day and this will promote weight loss [12]. Ho *et al.* [13] stated that, it is possible that different chemical compositions of chitosan could have variable effects on the binding of gastrointestinal lipids and thus weight loss. The risk of long-term ingestion of high doses of chitosan is that it could change the intestinal flora and allow the growth of unhealthful bacteria.Chitosan at a dose of 1,200 mg twice daily slightly reduced LDL cholesterol but did not affect total or HDL cholesterol levels [14]. However, Stokes *et al.* [3] found no improvement in cholesterol with 1,000 mg 3 times daily of a different chitosan product. Moreover, Polidori *et al.* [15] found that, in a seven-month study given 1,200 mg of chitosan daily has no beneficial effect on hyperlipidemia. Recently, it has also been reported that the addition of certain vitamins such as vitamin C to chitosan causes a larger increase in fecal fat excretion without affecting protein digestibility [16].

The purpose of the present study was to investigate the effects of Chitosan and mixture of Chitosan with certain vitamins (Vitamin C and Folic acid) on body weight, lipid metabolism and liver histolgy changes in normal rats and to increase the awareness of the health benefits that can be dreived from Chitosan

MATERIALS AND METHODS

Materials: Chitosan was extracted from shrimp shell, obtained from local market.

Methods: Chitin is extracted from shrimp/squid shells as follows:

The extraction of chitosan from shells basically included two stages namely demineralization followed by deprotienization.

- Demineralization: the shells were treated with Conc. HCl and CaCl2 salt was precipitated.
- Deproteinisation: the Amide bond is hydrolyzed in alkaline medium to give long chain aliphatic alcohol along with precipitation of Sodium carbonate.

The residue after Deproteinisation is Chitin, which is about 10% by weight of total weight of shells. Chitin Deacetylase = Chitosan

Process: The dry Shrimp shells are put in Conc., HCL. The treated shells are filtered and passed to dilute NaOH tank. The treated shells were dried in an open tank, or alternatively sun-dried.

Yield: Finally to obtain Chitosan, deacetylation was carried out by sodium hydroxide solution. The final product was white in color and insoluble in water [17]. Basic deacetylation provided 40% chitosan (89% purity), based on the dry weight of the shell powder [18].

Biological Evaluation

Diet: Standerd diet was prepared according to AIN [19].

Animals: Adult male albino rats Sprague Dawely strain weighing between (90-100) gm, were obtained from the animal house of the Egyptian Organization for Biological Products and Vaccines (VACSERA) Cairo, Egypt. The animals were kept in wire cages with wire bottom. The diet was introduced to the rats in special feed cup that kept food spilling to a minimum, water was provided to the rats by means of glass tube projecting through wire cage, an inverted bottle supported one side of the cage.

Experimental Design: Twenty four rats were divided into four groups: group (A) control fed on basal diet, group (B) fed on basal diet supplemented with 2% Chitosan, group (C) fed on basal diet supplemented with 2% chitosan + 1000 mg/ kg diet of Vitamin C according to Lisa *et al.* [20], group (D) fed on basal diet supplemented with 2% chitosan + 40 mg Folic acid / kg diet according to María *et al.* [21]. Rats were matched for body weight. All groups were fed the experimental diet for four weeks. At the end of experiment, animals were sacrified under ether anesthesia and blood samples were taken from the hepatic portal vein in centrifuge tubes to separate serum. Body weight and food intake were measured every day. The FER was calculated as daily weight gain (g) / daily dietary intake (g).

Biochemical Analysis: Determination of lipid parameters {Total cholesterol [22], high density lipoprotein cholesterol (HDL-C) [23], low density lipoprotein cholesterol (LDL-C) [24], very low density lipoprotein cholesterol (VLDL-C) [25] and triglycerides [26]. Serum total cholsterol was measured enzymatically by the cholesterol oxidase assay, whereas high – density lipoprotein cholesterol (HDL-C) was measured by the same procedure after precipitatin of low- denisty lipoprotein cholesterol (LDL-C) and very low- denisty lipoprotein –cholesterol (VLDL-C).

Statistical Analysis: The statistical analysis was conducted using one way analysis of variance technique (ANOVA) according to Sendecor and Cochran [27]. The significant difference among means evaluated by least significant difference (L.S.D) method, at levels of probability $P \leq 0.05$ and $P \leq 0.01$.

Histological Evaluation: After the animals were scarified, small pieces of the Liver, were taken and were fixed in 10% neutral formalin, the sections were stained with hematoxylin and eosin before being examined under a light microscope [28].

RESULTS AND DISCUSSION

From Table 1 and Fig. 1, it can be noticed that, there were a highly significant ($P \leq 0.01$) decreases in the body's weight of rats fed Chitosan+Vitamin C, followed by Chitosan+Folic acid and Chitosan group, when compared with the control group. It could be concluded that, feeding Chitosan resulted in reducing body weight of rats. Hirano [29] reported that, such a low weight gain was not caused by weight loss due to any toxicity of Chitosan, because it was widely accepted that Chitosan is a natural product with very low toxicity. Deuchi *et al.* [30] confirmed that, the epididymal fat pad was half-reduced in weight by ingesting Chitosan and concluded that, such a low weight gain is mainly attributable to a reduction in food efficiency, which is confirmed by the results obtained in the present study (Table 1).

Also, Chitosan binds fat in the intestine, blocking absorption and has been shown to lower blood cholesterol in animals and humans [31]. Reduction of fatty acids and bile acids will lead to less absorption of fat from the diets [32]. Since obesity is associated with numerous diseases, including diabetes, atherosclerosis and coronary heart disease among others, therefore, the findings of the effects of Chitosan feeding on body weight gain are of special significance [33]. Studies regarding the effects of Chitosan on overweight or obesity are still reporting inconsistent. Chitosan has been reported to have no effect on the reduction of body weight [34]. While, Bilheimer [35] found that a significant decrease in bodyweight. The results of this study was in agreement with the finding of Mhurchi *et al.* [36] who suggested that, the dietary Chitosan is more effective in reducing bodyweight gain in normocholesterolaemia, furthermore, the food intake of rats fed dietary Chitosan at 2% did not vary in normocholesterolaemic rats.

Results in Table 1 also illustrated relative organs weight (% liver weight /body weight), of rat groups fed on either the normal diet or diet containing chitosan supplemented with different vitamins. There was a non significant increase in the% liver weight /body weight, in Chitosan group. This result was in agreement with the result reported by Ikegami *et al.* [37] who suggested that, an increased bulk and viscosity in the intestinal contents considerably decreases the diffusion processes of substrates and enzymes and hinders their effective interaction of the mucosal surface, the animal compensates this inefficiency of nutrient digestion and absorption with an enlargement of digestive organs. The previous table showed that, there were no significant differences in food intake among all groups that was in agreement with the finding of Deuchi *et al.* [30]. Prusiewicz and Maciejewska [38] reported that, when different doses of Folic acid were given to rats, in the beginning there was a decrease in body weight and from the 7^{th} day the weight started to increase to reach control value in all groups on the 14^{th} day, food intake was deteriorating with increasing Folic acid concentration. Lam *et al.* [39] also found that Folic acid increases lipolysis in adipocytes and may have a role in the prevention of obesity. This mechanism involves the beta adrenoceptors in the adbdominal adipocytes. Folic acid supplements may reduce the accumulation of cholesterol in the liver and in the blood; this may be due to Folic acid's role in incorporating cholesterol into bile acid. Folic acid supplements have been shown to increase bile acid production and flow.

Table 1: Effect of dietary Chitosan2%, Chitosan 2% supplemented with 40 mg/kg diet of Folic acid and Chitosan supplemented with 1g/kg diet of Vitamin C, on body weight, food intake,%liver weight/body weight and Feed Effeiency Ratio (FER), (Mean± SE)

Group/ Parameter	Body Wt (gm/ week)	% liver/body Wt.	Food intake (gm/ week)	FER
Control	112.8±2.3	3.13±0.32	59.6±11.3	0.3±0.03
Chitosan	98.8±1.8**	3.87±1.45	59.4±11.7	-0.07±0.06**
Chitosan+Folic acid	92.2±1.2**	2.98±0.23	55.2±8.8	-0.15±0.05**
Chitosan+ Vitamin C	90.2±2.3**	3.1±0.23	66.7±6.8	-0.12±0.02**

** L.S.D at $P \leq 0.01$. - * L.S.D at $P \leq 0.05$.

Table 2: Effect of dietary Chitosan 2%, Chitosan 2% supplemented with 40 mg/kg diet of Folic acid and Chitosan supplemented with 1g/kg diet of Vitamin C, on Serum Lipids' Fraction, (Mean ±SE)

Group/ Parameter	Total cholesterol mg/dl	Triglycerides mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
Control	77.38±0.59	98.22±1.21	35.38±0.78	22.16±0.16	19.81±0.18
Chitosan	80.90±1.85	111.92±0.56**	35.39±1.02	23.12±1.27	22.38±0.11**
Chitosan+Folic acid	77.87±1.98	95.55± 2.1	35.70±2.07	23.06±1.11	19.11±0.43
Chitosan+Vitamin C	76.87±1.17	91.15±2.46**	36.53±1.46	21.34±2. 6	18.23±05**

** L.S.D at P≤ 0.01. - * L.S.D at P≤0.05

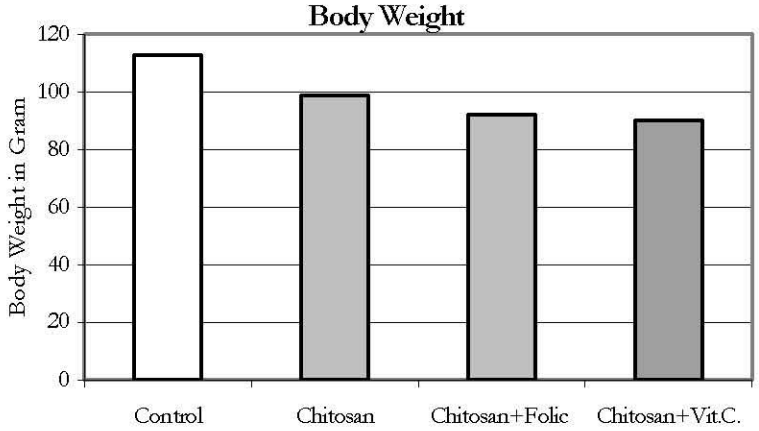

Fig. 1: Effect of Chitosan 2%, Chitosan 2% supplemented with 40 mg/ kg diet Folic acid and Chitosan supplemented with 1g/kg diet Vitamin C., on body weight.

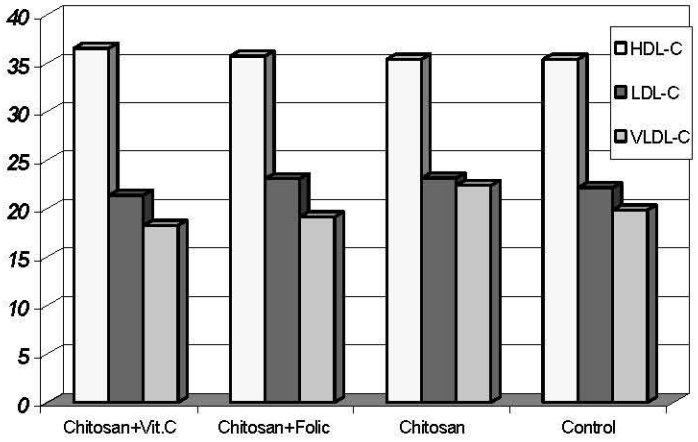

Fig. 2: Effect of dietary Chitosan2%, Chitosan 2% supplemented with 40mg/kg diet of Folic acid and Chitosan supplemented with 1g of Vitamin C on Serum HDL-C, LDL-C and VLDL-C.

Data in Table 2 showed that, there was a non significant decrease in serum total Cholesterol of rats fed on Chitosan+Vitamin C (76.87mg/dl), while, rat fed on Chitosan showed non significant increase in serum cholesterol (80.90 mg/dl) when compared with contol group (77.38 mg/dl). Fig. 3 showed that, there was a highly significant (P≤ 0.01) decrease in serum triglycerides of rats fed on Chitosan+Vitamin C (91.15 mg/dl), when compared with control group (98.22 mg/dl), whereas, there was a highly significant (P≤ 0.01) increase in serum triglycerides of rats groups fed on Chitosan 2% (111.92 mg/dl), when compared with control group (98.22 mg/dl). The HDL-C tended to be increased in groups fed on (Chitosan+Folic acid) and (Chitosan+Vitamin C), but the differences were not significant when compared with control group. On the other hand, LDL-C tended to be decreased in the group fed on Chitosan supplemented with Vitamin C, although the differences were not significant.

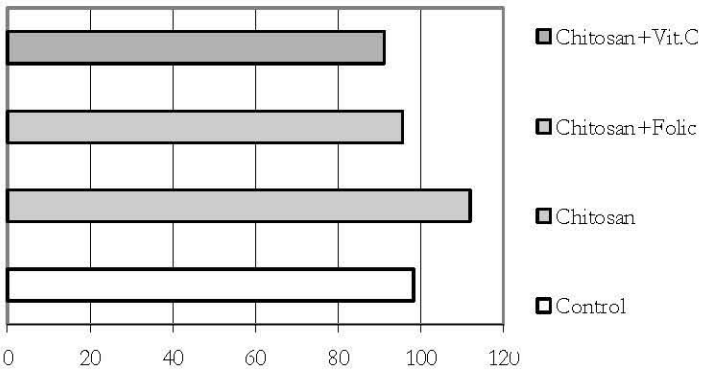

Fig. 3: Effect of dietary Chitosan 2%, Chitosan 2% supplemented with 40mg/kg diet of Folic acid and Chitosan supplemented with 1g of Vitamin C on Serum Triglycerides.

There was a highly significant ($P \leq 0.01$) decrease in serum VLDL-C of rats fed on Chitosan+Vitamin C (18.23 mg/dl), when compared with that of the control group (19.81mg/dl). These results of Chitosan+Vitamin C, were in agreement with that of Kanauchi [40], who reported that, Vitamin C serves to enhance and augment the adsorbability of lipids, in other words, it makes the fat binding action of Chitosan more complete, moreover the clinical study have discovered that supplementing Chitosan with Vitamin C resulted in even less fat digestion and absorption in the intestines, the addition of Vitamin C almost doubles Chitosan's effectiveness as a fat blocker. Recently, Tsujikawa *et al.* [15] reported that, the addition of ascorbic acid to Chitosan causes a larger increase in fecal fat excretion without affecting protein digestibility.

It has been noted that, the mixtures of Chitosan with Folic acid or Vitamin C, were more effective in lowering the LDL-C and VLDL-C, than Chitosan alone. The best reduction in LDL-C and VLDL-C levels, were noticed in the groups fed on the mixtures of Chitosan with vitamins. The levels of total cholesterol, triglycerides and VLDL-C were decreased, whereas HDL-C was not significantly changed, vitamins mixtures supplemented diets were more effective against hypercholesterolemia than Chitosan alone [41]. Gallaher *et al.* [31] stated that, Chitosan binds fat in the intestine, blocking absorption and has been shown to lower blood cholesterol in animals and humans. The reduction of fatty acids and bile acids will lead to less absorption of fat from the diets and the reduction of endogenous cholesterol due to the interruption of enterohepatic bile acid circulation. This will influence cholesterol metabolism. Moreover, Zhou *et al.* [7] stated that, Chitosan is soluble in the acidic conditions of the stomach and forms a gel when the molecular weight is high. When fat and Chitosan in the diets are eaten together, the viscous Chitosan will entrap the fat droplet in the stomach. In the small intestine (neutral pH), Chitosan forms a precipitate and prevents the digestion of fat. Tsujikawa *et al.* [15] speculated that, gastric acid–soluble Chitosan mixed with dietary fat in the stomach, with the emulsifying process is effectively mediated by ascorbic acid. Vitamin C supplementation provided a significant reduction in both LDL cholesterol and triglycerides [42, 43]. These results confirm the results of the present study, which indicated that, Chitosan with Vitamin C, has more lowering effect of Serum lipid profile than chitosan alone. Polidori *et al.* [13] showed that, Vitamin C is able to intercept reactive oxygen species in the aqueous phase of plasma, thereby significantly reducing plasma lipid peroxide levels and thus inhibiting oxidative modification of LDL-C. Ascorbic acid's antioxidant protection of very low-density lipoprotein may therefore facilitate its uptake by the liver and hence promote its removal from the plasma. Furthermore, it was reported that, Vitamin C stimulates fatty acids utilization in hepatocytes by enhancing carnitine synthesis. Vitamin C is required as a cofactor in hydroxylation reactions in the pathway of carnitine biosynthesis. If hepatic carnitine concentration increased, that results in a further hepatic fatty acid β-oxidation, then as a result, there will be a reduction in the plasma triglyceride concentration [43]. Ekuni *et al.* [44] stated that, lipid peroxidation is implicated in the development of atherosclerosis and the chemical and biological properties of Vitamin C as an antioxidant *in vivo*, will protects against oxidation of isolated LDL, primarily by scavenging reactive oxygen species in the aqueous milieu. Vitamin C may improve the treatment of atherosclerosis with decreasing lipid peroxidation and increasing antioxidant level. It was hypothesized that, vitamin C may even have a hypocholesterolemic effect, because the enzyme needed

for the first step in bile acid synthesis (cholesterol 7-alpha hydroxylase), is dependent upon the presence of vitamin C [45]. Moreover, Vitamin C prevents the oxidative modification of LDL and provides protection against lipid peroxidation [46]. Vitamin C inhibits lipid oxidation in HDL and preserves the antioxidants activity associated with this lipoprotein fraction, which is an important risk factor for atherosclerosis [47].

The mixtures of Chitosan with vitamins insure a more complete solubilization of the Chitosan under gastric conditions. The increased solubilization increases the Chitosan surface area and its initial intestinal viscosity as it enters the upper gastrointestinal tract. Greater intestinal viscosity enhances the hypocholesterolemic effect of unabsorbable fibers, which is reflected in blood serum. In addition, very high concentration of ascorbic acid or its sodium salt enhances Chitosan's fat binding, as reflected by increased fat excretion. This effect could be due to other factors caused by the very high level of Vitamin C in the diet of experimental animals [48]. Hirano [29] showed that, *in vitro*, Chitosan bind and precipitate 4–5 times its weight in micella lipids including bile salts, cholesterol and triglyceride. Chitosan is proposed to bind bile acids with ionic bonds in the same manner as cholestyramine [49]. The deacetylation of chitin to form chitosan increases the positive charge density on the molecule, permitting it to form these bonds [49]. Studies in rats, [50, 51] and in humans [52] suggested that, dietary Chitosan increases bile acid excretion and decreases plasma cholesterol. The apparent fat digestibility of a defined diet containing a high dose of Chitosan (5% w=w) fed to rats was 51.22% compared to 95.2%, using a cellulose-containing control diet. In another study, Bilheimer [35] stated that, the use of a high viscosity Chitosan (5% w=w) in rats' diet, also reduced the apparent fat digestibility to about 50%. Based on the aforementioned studies in rats, the consumption of 28 g of Chitosan daily, only 50% (less than 70 g of the 135 g of dietary fat eaten), would be excreted. Several studies have shown that, cholesterol-lowering effect of Chitosan is related to its dietary levels and particle size [53]. This may explain why chitosan at 2% in our study is likely to be less effective in reducing total cholesterol. Schiller *et al.* [54] pointed that, after 4 weeks of treatment, total serum cholesterol was not significantly different in subjects receiving Chitosan compared to those receiving placebo. They also indicated that, total serum cholesterol did not change significantly after 4 weeks of Chitosan treatment, however a significant decrease was observed after 8 weeks. Triglycerides were slightly increased with the use of Chitosan. These findings suggested that, the beneficial effect of Chitosan may appear after 8 weeks, perhaps because Chitosan does not reduce cholesterol synthesis, but reduces lipid absorption from the gastrointestinal tract. Marlett [55] suggested that, Chitosan at 5% of the diet was recommended deservingly for long-term feeding test in rat. Although how Chitosan reduced cholesterol was still uncertain. Many studies indicated that, increased bile acid excretion and/or decreased cholesterol absorption was responsible. Chitosan acts as a weak anion exchange resin and exhibits a substantial viscosity *in vitro*. Either of these properties of Chitosan could mediate its hypocholesterolemic effect. However, Sugano *et al.* [56] found that, Chitosan preparations of different viscosities demonstrated equivalent hypocholesterolemic effects, arguing against a role for viscosity. The anion exchange property of chitosan would seem to be favored as an explanation for its hypocholesterolemic properties and noted that there is an increase in cholesterol excretion in rats fed 5% chitosan, compared to cellulose treatment and a change in the composition of the fecal sterols resulted in excreting relatively more cholesterol and less coprostanol in rats treated with Chitosan. In their study, they also found a strong trend to a decrease of bile acid excretion in rats fed 5% chitosan, compared to cellulose. Increased bile acid excretion could reduce cholesterol concentrations because plasma or liver cholesterol would be utilized to maintain the bile acid pool. Difference of LDL receptor mRNA abundance in some animals such as rabbits and guinea pigs could be correlated with differences in plasma total and LDL cholesterol concentrations [57]. Guangfei *et al.* [52] found that, Chitosan increased hepatic LDL receptor mRNA expression compared to cellulose. The levels of hepatic LDL receptor mRNA in rats fed cholesterol enriched with 5% Chitosan even were greater than those of rats fed cholesterol-free diet. Lehoux and Grondin [9] illustrated that, the liver plays a central role in lipoprotein metabolism besides the production of several apolipoproteins; the liver also produces enzymes and receptors involved in lipoprotein metabolism such as HMG CoA reductase and the low density lipoprotein (LDL) receptor. HMG CoA reductase is the ratelimiting enzyme in endogenous sterol biosynthesis. This enzyme's activity in rats fed a Chitosan-sterol diet was lower than in those fed normal diet, although, HMG CoA reductase and mRNA levels were normal. Inhibition of pancreatic lipase within the small intestine would lead to accumulation of a lipid emulsion leading to greater excretion of cholesterol. This concept is consistent with the finding that administration of the pancreatic lipase inhibitor tetrahydrolipstatin to mice reduced cholesterol absorption [58].

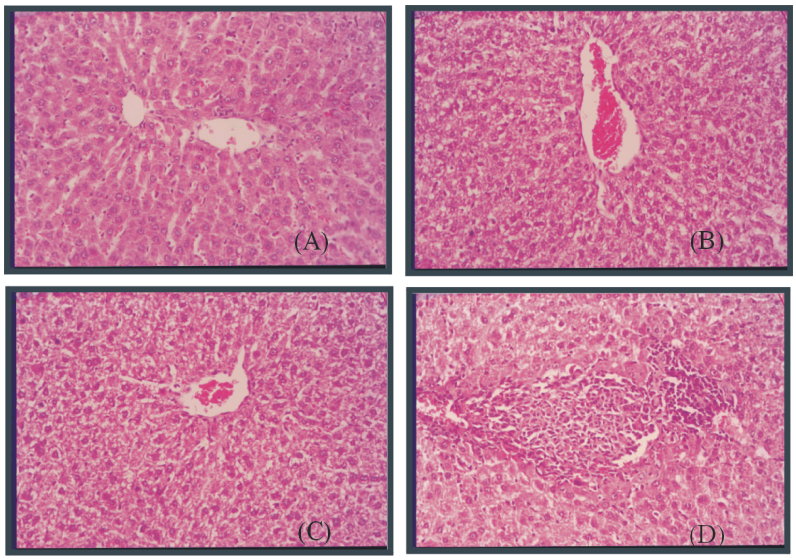

Fig. 4: Histopathological changes detected in the liver of normal control group, (B) Chitosan group, (C) Chitosan + Folic acid and (D) Chitosan + Vitamin C.

Histopathological Results: The histological observations of liver in the normal and experimental groups are presented in Fig.4. No histological changes were observed in the liver of control group (Fig.4A). On the contrary, liver of rats fed on Chitosan, showed slight congestion of central vien associated with vacuolations of hepatocytes (Fig.4B). Liver of rats fed on Chitosan+Folic acid showed vacuolations of hepatocytes (Fig.4C). Meanwhile, liver of rats fed on Chitosan+Vitamin C showing focal area of hepatic necrosis associated with leucocytic cells infiltration (Fig.4D). Yoshinori *et al*. [59] showed that, when 5 mg of Chitosan were injected intraperitoneally, the body weights of the mice decreased significantly and inactivity was observed in the fifth week. Histologically, many macrophages with hyperplasia were observed in the mesenterium. Finally, further research is required to study the effect of this by- product on body organs.

In conclusion, special care should be taken in the use of Chitosan over a long time and further studies are needed regarding this point.

REFERENCES

1. Bhatnagar, D., H. Soran and P.N. Durrington, 2008. Hypercholesterolaemia and its Management. BMJ, 337.
2. Anderson, J.W., P. Baird, R.H. Davis, S. Ferreri, M. Knudtson, A. Koraym and V. Waters, 2009. Health benefits of dietary fiber. Nutr. Rev., 67(4): 188-205.
3. Stokes, K.Y., D. Cooper, A. Tailor and D.N. Granger, 2002. Hypercholesterolemia Promotes Inflammation and Microvascular Dysfunction: Role of Nitric Oxide and Superoxide. Free Radical Biology Medicine, 33: 1026-1036.
4. Sudhahar, V., S.A. Kumar, P.T. Sudharsan and P. Varalakshmi, 2007. Protective Effect of Lupeol and its Ester on Cardiac Abnormalities in Experimental Hypercholesterolemia. Vascul. Pharmacol., 46: 412-418.
5. Muzzarelli, R.A.A, P. Ilari and M. Petrarulo, 1994. Solubility and Structure of *n*-Carboxy Methylchitosan. Int. J. Biol. Macromol., 16: 177-80.
6. Muzzarelli, R.A.A., 1996. Chitosan-Based Dietary Foods. Carbohydr. Polym., 29: 309-316.
7. Liu, J., J. Zhang and W. Xia, 2008. Hypocholesterolaemic Effects of Different Chitosan Samples *In vitro* And *In vivo*. Food Chem., 107: 419-425.
8. Zhou, K., W. Xia, C. Zhang and L. Yu, 2006. *In vitro* Binding of Bile Acids and Triglycerides by Selected Chitosan Preparations and Their Physicochemical Properties. LWT-Food Sci. Technol., 39: 1087-1092.
9. Ventura, P., 1996. Lipid Lowering Activity of Chitosan, A New Dietary Integrator. In: Muzzarelli RAA(Ed).Chitin Enzmology, Vol.2.Atec Edizioni, Ancona., 55-62.
10. Lehoux, J.G. and F. Grondin, 1993. Some Effects of Chitosan on Liver Function in the Rat. Endocrinol., 132: 1078-84.

11. Peacock, J.L., R.I. Inculet, R. Corsey, D.B. Ford, W.F. Rumble, D. Lawson and J.A. Norton, 1987. Resting Energy Expenditure and Body Cell Mass Alterations in Noncachectic Patients with Sarcomas. Surgery (St. Louis), 102: 465-473.
12. Papakonstantinou, E., W.P. Flatt, P.J. Huth and R.B. Harris, 2003. High Dietary Calcium Reduces Body Fat Content, Digestibility of Fat and Serum Vitamin D in Rats. Obes. Res., 11(3): 387-94.
13. Ho, S.C., E.S. Tai, P.H. Eng, C.E. Tan and A.C. Fok, 2001. In the absence of dietary surveillance, chitosan does not reduce plasma lipids or obesity in hypercholesterolaemic obese Asian subjects. Singapore Med. J., 42: 6-10.
14. Bokura, H. and S. Kobayashi, 2003. Chitosan Decreases Total Cholesterol in Women: A Randomized, Double-Blind, Placebo-Controlled Trial. Eur. J. Clin. Nutr., 57: 721-5.
15. Polidori, M.C., P. Mecocci, M. Levine and B. Frei, 2004. Short-Term and Long-Term Vitamin C Supplementation in Humans Dose dependently Increases the Resistance of Plasma to *Ex vivo* Lipid Peroxidation. Arch. Biochem. Biophys., 423(1): 109-115.
16. Tsujikawa, T., O. Kanauchi, A. Andoh, T. Saotome, M. Sasaki, Y. Fujiyama and T. Bamba, 2003. Supplement of A Chitosan and Ascorbic Acid Mixture for Crohn's Disease: A Pilot Study. Nutrition, 19: 137-139.
17. Roshana, N., 2005. Extraction of Chitin and Chitosan from Shrimp (*Metapenaeus monoceros*) Shell by Chemical Method. Pakistan J. Biological Sci., 8(7): 1051-1054.
18. Sahdat, H., R. Aizur, K. Yearul, A. Ali, F. Fahmida and H. Mchio, 2007. Effects of Shrimp (*Macrobracium rosenbergii*)-Derived Chitosan on Plasma Lipid Profile and Liver Lipid Peroxide Levels in Normo- and Hypercholesterolaemic Rats. Clinical and Experimental Pharmacol. and Physiol., 34(3): 170-176.
19. American Nutrition Institute (AIN), 1993. Purified Diet For Laboratory Rodent: G. Philip, F. Reevs, H. Nielsen, C. George and J.R. Fahey. J. Nutr. 123: 1939-1951.
20. Lisa, A.B., M.M. Gerard, R.W. Grillian, M.H. Bernadette and A.B. Yvonne, 2000. The effect of vitamin C or vitamin E supplementation on based and H2O2 induced DNA damage in human lymphpcytes. British. J. Nutr., 84: 195-202.
21. María, R., A. María, M. Félix, R. Carmen, V. Gregorio and P.M. Julia, 2004. Folic Acid Supplementation for 4 Weeks Affects Liver Morphology in Aged Rats. J. Nutr., 134: 1130-1133.
22. Stein, E.A., 1986. Enzymatic determination of total chalesterol in serum. In Textbook of Clinical Chemistry, pp: 879-886: 1818-1829.
23. Wieland, H. and D. Seidel, 1981. HDL. Cholesterol determination after separation of high density lipoprotein. Art Ztl. Lab., 27: 141-154.
24. Frunchart, J.C., 1982. LDL-cholesterol determination after separation of low-density lipoprotein. Rev. Fr. Des Laboratories, 103: 7-17.
25. Fniedewald, W.T., R.I. Leve and D.S. Fredrickson, 1972. Estimation of concentration of low-density lipoproteins separated by three different methods. Clin. Chem., 18: 499-502.
26. Scheletter, G. and E. Nussel, 1975. Quantitative enzymatic determination of triglycerides in serum. Arbilsmed Sozialrned Pracentirned, 10-25.
27. Sendecor, G.W. and W.G. Cochran, 1967. Statistical Methods. 6th Edition, Iowa State University Press, Ames., U.S.A.
28. Degertekin, H., K. Aldamar, R. Yates, I. Chen, A. Ertain and R. Vauple, 1986. Light and Elctron Microscopic Studies of Diet Induced Hepatic Changes In Mice. Acta Anat (Basel), 125: 174-179.
29. Hirano, S., 1996. Chitin Biotechnology Applications. Biotechnol. A Rev., 2: 237-258.
30. Deuchi, K., O. Kanauchi, Y. Imasato and E. Kobayashi, 1994. Decreasing Effect of Chitosan on The Apparent Fat Digestibility by Rats Fed on A High Fat Diet. Biosci. Biotech. Biochem., 58: 1613-16.
31. Gallaher, C.M., J. Munion, J. 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: 2753-2759.
32. Maezaki, Y., T. Keisuke, Y. Nakagawa, Y. Kawai, M. Akimoto, T. Tsugita, W. Takekawa, A. Terada, H. Hara and T. Mitsuoka, 1993. Hypocholesterolemic Effect of Chitosan in Adult Males. Biosci Biotechnol Biochem., 59: 1439-1444.
33. Nunthanid, J., M. Laungtanan, P. Siramornsak, S. Limmatvapirat, S. Puttippatkhachorn, L.Y. Lim and E. Khor, 2004. Characterization of Chitosan Acetate as A Binder for Sustained Release Tablets. J. Control. Release, 99: 15-26.
34. Kanauchi, O., K. Deuchi, Y. Imasato, M. Shizukuishi and E. Kobayashi, 1995. Mechanism for the Inhibition of Fat Digestion by Chitosan and for the Synergistic Effect of Ascorbate. Biosci. Biotechnol. Biochem., 59: 786-90.
35. Bilheimer, D.M., 1988. The Lipoprotein Receptor Concept. Drugs, 36: 55-62.

36. Mhurchi, C.N., C. Dunshea-mooji, D. Bennett and A. Rodgers, 2005. Effect of chitosan on weight loss in overweight and obese individuals: A systematic review of randomized controlled trials. Obese Rev., 6: 3-4.
37. Ikegami, S., F. Tsuchihashi, H. Harada, N. Tsuchihashi, E. Nishide and S. Innami, 1990. Effect of viscous indigestible polysaccharides on pancreatic biliary secretion and digestive organs in rats. J. Nutr., 120: 353-360.
38. Prusiewicz-Witaszek, U. and M. Maciejewska, 1994. Effect of various amounts of Folic acid added to rat food on food intake and some parameters of carbohydrate metabolism. Arch. Vet. Pol., 34(3-4): 261-6.
39. Lam, T.Y., S.W. Seto, A.L. Au, C.C. Poon, R.W. Li, H.Y. Lam, W.S. Lau, S.W. Chan, S.M. Ngai, G.P. Leung, S.M. Lee, S.K. Tsui and Y.W. Kwan, 2009. Folic acid supplementation modifies beta-adrenoceptor-mediated *in vitro* lipolysis of obese/diabetic (+db/+db) mice. Experimental Biology and Medicine (Maywood, N.J.), 234(9): 1047-55.
40. Kanauchi, O., K. Deuchi, Y. Imasato, M. Shizukuishi and E. Kobayashi, 1995. Mechanism for the Inhibition of Fat Digestion by Chitosan and for the Synergistic Effect of Ascorbate. Biosci Biotechnol Biochem., 59: 786-790.
41. Yao, H.T., S.Y. Huang and M.T. Chiang, 2008. A Comparative Study on Hypoglycemic and Hypocholesterolemic Effects of High and Low Molecular Weight Chitosan in Streptozotocin-Induced Diabetic Rats. Food Chem. Toxicol., 46: 1525-1534.
42. Mcrae, M.P., 2007. Vitamin C Supplementation for Treating Hyper-cholesterolemia: A Meta-Analysis of 16 Randiomized Controlled Trials. J. Am. Nutraceut. Ass., 10(2): 21-28.
43. Mcrae, M.P., 2008. Vitamin C Supplementation Lowers Serum Low-Density Lipoprotein Cholesterol and Triglycerides: A Meta-Analysis of 13 Randomized Controlled Trials. J. Chiropr. Med., 7: 48-58.
44. Ekuni, D., T. Tomofuji, T. Sanbe, K. Irie, T. Azuma, T. Maruyama, N. Tamaki, J. Murakami, S. Kokeguchi and T. Yamamoto, 2009. Vitamin C Intake Attenuates the Degree of Experimental Atherosclerosis Induced by Periodontitis the rat by decreasing oxidative stress. Archives of Oral Biol., 54(5): 495-502.
45. Jacob, R.A., 1999. Vitamin C. In: Modern Nutrition in Health and Disease. Ninth Edition. Edited by Maurice Shils, James Olson, Moshe Shike and A. Catharine Ross. Baltimore: Williams and Wilkins., 467-482.
46. Mandeep, R.M., J.L. Carl, O.V. Hector and V.M. Richard, 1995. Prevention of atherosclerosis: the potential role of Antioxidants. Postgrad Med., 98(1): 175-176.
47. Hillstorm, R.J., K. Angela, A. Yacapin and M.L. Sean, 2003. Vitamin C inhibits lipid oxidation in human HDL. J. Nutr., 133: 3047-3051.
48. Furda, I., 1998. Multifunctional fat absorption and blood cholesterol reducing formulation comprising chitosan United States Patent, 5736532.
49. Furda, I. and C.J. Brine, 1990. American Chemical Society Meeting. New Developments in Dietary Fiber: Physiological, Physicochemical and Analytical Aspects. Plenum Press: New York.
50. Fukada, Y., K. Kimura and Y. Ayaki, 1991. Effect of Chitosan Feeding on Intestinal Bile Acid Metabolism in Rats. Lipids, 26: 395-399.
51. Ormrod, D.J., C.C. Holmes and T.E. Miller, 1998. Dietary Chitosan Inhibits Hypercholesterolaemia and Atherogenesis in the Apolipoprotein E- Deficient Mouse Model of Atherosclerosis. Atherosclerosis, 138: 329-334.
52. Jing, S.B., L. Li, D. Ji, Y. Takiguchi and T. Yamaguchi, 1997. Effect of Chitosan on Renal Function in Patients with Chronic Renal Failure. J. Pharm. Pharmac., 49: 721-723.
53. Guangfei, X.M., H.B. Xiaodong, Q.M. Lianglin, W.M. Jinbiao and H.B. Yinqing, 2007. Mechanism Study of Chitosan on Lipid Metabolism in Hyperlipidemic Rats. J. Clin. Nutr., 16(Suppl 1): 313.
54. Schiller, R.N., E. Barrager, A.G. Schauss and E.J. Nichols, 2001. A randomized, double-blind, placebo-controlled study examining the effects of a rapidly soluble Chitosan dietary supplement on weight loss and body composition in overweight and mildly obese individuals. J. Am. Nutraceut Assoc., 4: 42-49.
55. Marlett, J.A., 1997. Sites and mechanisms for the hypocholesterolemic actions of soluble dietary fiber sources. Adv. Exp. Med. Biol., 427: 109-121.
56. Sugano, M., S. Watanabe, A. Kishi, M. Izume and A. Ohtakara, 1988. Hypocholesterolemic action of chitosan with different viscosity in rats. Lipids, 23: 187-191.

57. Ramjiganesh, T., S. Roy, H.C. Freake, J.C. McIntyre and M.L. Fernandez, 2002. Corn fiber oil lowers plasma cholesterol by altering hepatic cholesterol metabolism and upregulating LDL receptors in guinea pigs. J. Nutr., 132: 335-340.
58. Young, S.C. and D.Y. Hui, 1999. Pancreatic lipase/colipase-mediated triacylglycerol hydrolysis required for cholesterol transport from lipid emulsions to intestinal cells. Biochem. J., 339: 615-620.
59. Yoshinori, T., T. Shin-chiro, T. Miyoko, T. Takahiko, K. Yukisato, M. Saburo, O. Yoshiharu, M. Mariko and N. Masanobu, 1997. Effects of chitin and chitosan particles on BALB/c mice by oral and parenteral administration. Biomaterials., 18(8): 591-595.