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Hypolipidemic effects of chitosan isolated from shrimp waste in rats fed with high fat diet

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ABSTRACT

Chitosan is a natural polysaccharide with non-toxic, biodegradable, and biocompatible properties. Subsequently, there has been much interest in chitosan and it has been widely used in many fields. Chitosan, which has high in dietary fiber, is thought to have Hypolipidemic effects. Chitosan was prepared and characterized using X-ray Diffractometry (XRD) and Fourier Transform Infrared Spectroscopy (FT-IR). The present study was performed to further elucidate the Hypolipidemic action of chitosan for their effects on the diet-induced hyperlipidemia in rats. It has been reported that plasma cholesterol and triglyceride concentrations decrease when animals are fed with chitosan. Rats were fed a cholesterol- free diet , (negative control) , cholesterol-enriched diet and 5% of lard for 6 weeks. The study investigated the effects of chitosan on triglyceride , total cholesterol, HDL-C and LDL-C levels behinds effect of chitosan on AST and ALT enzymes , protein and albumin concentrations . The results showed that chitosan could decrease levels of triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and increase (HDL-C) in plasma (p<0.05). In addition chitosan could decrease levels of AST and ALT enzymes but the levels of total protein and albumin in plasma were unchanged (p<0.05).

Keywords: Chitosan - cardiovascular disease - dyslipidemia - hypercholesterolemia.

1. INTRODUCTION

Serum lipid abnormalities result in increasing vascular risks; hence, aggressive treatment of hyperlipidemia is recommended. Advancing age and hypercholesterolemia have been widely considered cardiovascular risk factors in the elderly **(Davidson et al. 2003)**. Chitosan shows (Figure 1), a natural polysaccharide of β-1,4-linked glucosamine residues, is a biopolymer obtained primarily from the exoskeletons of crustaceans. Hydrolysis of chitin from shrimps and crabs results in deacetylation of the aminoacetyl groups and yields chitosan **(Muzzarelli et al, 1994)**. Although it is not derived from plants, it shares the same characteristics as dietary fiber, which is a indigestible polysaccharide by mammalian digestive enzymes. It is well known that chitosan is the only abundant cationic polymer having an amino group in its chemical structure **(Maezaki et al., 1993; Muzzarelli, 1996)**, The degree of deacetylation (DD) is a characteristic greatly different from those of other dietary fibres. Higher DD means that there are more free amino groups in the chitosan molecule and more positive charge in chitosan solution.

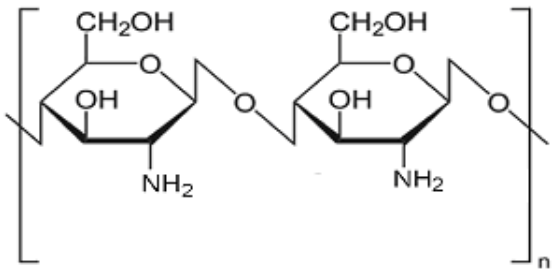

Figure - 1: Chemical and 3D-structure of N-Dglucosamine.

It was reported that the hypocholesterolaemic activity of chitosan was better when DD was high (90% deacetylated), which might be due to the electrostatic force between chitosan and anion substances, such as fatty acid and bile acid **(Deuchi, Kanauchi, Imasato and Kobayashi, 1995; Vahouny, Satchithanandam, Cassidy, Lightfoot, and**

Furda, 1983). Dyslipidemia, including hypercholesterolemia, hypertriglyceridemia, or their combination, is a major risk factor for cardiovascular disease. Generally, dyslipidemia is characterized by increased fasting concentrations of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), in conjunction with decreased concentrations of high-density lipoprotein cholesterol (HDL-C) **(Varady and Jones 2005).** 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 3-hydroxy-3-methylglutaryl coenzyme- A (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 more elevated than in those fed a sterol diet but lower than in those fed normal diet, **(Lehoux and Grondin 1993).** The specific function of LDL receptor is to remove cholesterol-rich lipoprotein particles from the circulation , which is a highly regulated pathway that has been shown to be down-regulated by dietary cholesterol in experimental animals and in humans. **(Brown and Goldstein 1986 and Kovanen et al. 1981).** When ingested, chitosan develops an HCl-layer in the stomach. As capsulated particles of chitosan move into the duodenum, the HCl-layer becomes diluted and the chitosan particles form agglomerates with fatty acids and cholesterol, thus reducing lipid absorption from the gastrointestinal tract. Studies in primates have shown that chitosan can increase the amount of fat eliminated in the stool, this finding led to the use of chitosan as a dietary supplement for weight loss or serum cholesterol reduction. **(Sugano et al, 1980; Ebihara and Schneeman, 1989)**. Chitosan has been suggested to reduce fat absorption from gastrointestinal tract by binding with anionic carboxyl groups of fatty and bile acids, and it interferes with emulsification of neutral lipids (i.e., cholesterol, other sterols) by binding them with hydrophobic bonds **(Ylitalo et al. 2002).** The hypolipidemic influence of chitosan may also be due to interruption of the enterohepatic bile acid circulation , and the reduction in duodenal bile acid concentration **(Razdan and Pettersson 1996 and Razdan et al. 1997)**. The molecular weight of chitosan is proportional to its viscosity and either of these two parameters can represent the size of the molecule. The entrapment caused by a viscous polysaccharide, which would reduce the absorption of fat and cholesterol in the diet, was thought of as the hypocholesterolaemic mechanism of dietary fibres (**Kanauchi, Deuchi, Imasato, Shizukuishi,& Kobayashi, 1995)** The hypocholesterolaemic action of chitosans in rats was independent of their molecular weight within the tested viscosity range 30–1620 cps, and the effect of chitosan on the apparent fat digestibility in rats was greater as its viscosity increased **(Sugano et. al. 1988**, **and Deuchi et al., 1995)**. Another report found that high molecular weight chitosans (>750 kDa) were less effective as hypocholesterolaemic agents than a 70 kDa preparation **(LeHoux & Grondin, 1993).** The primary objective of this study was to determine the effect of two different doses of chitosan (300 and 450 mg chitosan / L drink water) on plasma triglycerides, total cholesterol , LDL and HDL cholesterol concentrations to understand how chitosan lower cholesterol. The effect of chitosan on fat excretion was also determined as a possible explanation for the observation in several studies that chitosan supplements accelerated weight loss in subjects consuming hypocaloric diets (**Sciutto and Colombo 1995, Veneroni *et al*., 1996 and Gallaher *et al*., 2000)**

2. MATERIALS AND METHODS

2.1. Production of chitosan

The production of chitosan involved the demineralization (DM), deproteinization (DP), and deacetylation (DA) steps **(Youn et al., 2007)**. The shrimp shell was demineralized with 1 mol/L HCl for 30 min at ambient temperature with a solid/solvent ratio of 1:15 (w/v). Following the DM step, the demineralized shell was collected on a 100-mesh sieve, washed to neutrality in running tap water, rinsed with deionized water, and filtered to remove excess moisture. The DP step was accomplished by treating the demineralized shell with 3 g/100 mL NaOH for 15 min at 15 psi/ 121 ºC and a solid/solvent ratio of 1:10 (w/v). The residue was then washed , filtered as mentioned above, and dried at 60 ºC for 4 h in a forced-air oven. The DA step was achieved by treating chitin under conditions of 15 psi/121 ºC with 45 g/100 mL NaOH for 30 min and a solid/solvent ratio of 1:10 (w/v). The resulting chitosan was collected, washed as mentioned above, and dried at 60 ºC for 4 h in a forced-air oven.

2.2. Determination of the deacetylation percent

Chitosan (0.5 g) was dissolved in 25 ml of 0.1 M standard HCl aqueous solution. The solution was then toped up to 100 ml with distilled water and calculated amount of KCl was added to adjust the ionic strength to 0.1 M. The titrant was a solution of 0.05 M NaOH. The pH meter was used for pH measurements under continuous stirring. The titrant was added until the pH value reached 2.00, the standard NaOH was then added stepwise

and the pH values of solution were recorded and a curve with two inflection points was obtained. The difference of NaOH solution volumes between these points corresponds to the acid consumed for salification of the amine groups of chitosan and allows the determination of DDA% of the chitosan. The DDA was calculated from the relation **(Broussignac, 1968).**

$$DDA\ \% = (1\text{-}161\ Q)/(1+42\ Q)$$

Where Q = NDV/m, DV is the volume of NaOH solution between the two inflection points, N is the concentration of NaOH (0.05 mol/l^{-1}) and m is the dry weight of chitosan g.

2.3. Characterization of chitosan

2.3.1. Fourier transform infrared spectroscopy (FT-IR)

IR spectra of chitosan obtained from shrimp shells was recorded with a Tensor 27 Fourier transform infrared spectrometer FTIR. The spectral region between4000 and 400 cm^{-1} was scanned (Figure 2). Specimens prepared as KBr pellets were used. Dried, powder chitosan was mixed thoroughly with KBr and then pressed in vacuo to homogeneous disc with a thickness of 0.5 mm. The chitin concentration in the sample was calculated with respect to KBr.

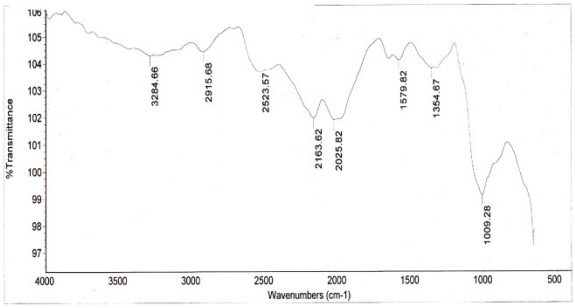

Figure - 2: Shows Fourier transform infrared spectroscopy spectra of chitosan.

2.3.2. X – Ray Diffraction (XRD)

X–ray diffraction (XRD) patterns of chitosan was studied using X-ray powder diffractometer (XRD – SHIMADZU XD – D1) using a Ni – filtered Cu Kα X–ray radiation source. The relative intensities were recorded within the range of 10º – 90º (2θ) at a scanning rate of 5º min^{-1}

2.4. Biological Evaluation

2.4.1. Diet

Standard diet was prepared according to AiN 1993.

2.4.2. Animals

Adult male albino rats Sprague Dawely strain weighing between (90 – 100) gm , were obtained from the animal house of 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.

2.4.3. Experimental Design:

Twenty rats were divided into four groups: group (A) control fed on basal diet, groups (B , C and D) were allowed to feed hyperlipidemic diet to induce hyperlipidemic through the feeding period. One of each experiment continued feeding hyperlipidemic diet without any supplementation saved as hyperlipidemic group (B) and the other two groups of each experiment were allowed to feed hyperlipidemic diet with 300 mg / L chitosan in drink water as group (C) and 450 mg / L chitosan in drink water as group (D). Standard diet composition was described in (Table 1) by (**Campbell., 1961**) and hyperlipidemic diet was described by (**Nakamura et al., 1989**) as follows:

Table - 1: Standard and hyperlipidemic diets

Ingredient	Standard diet	Hyperlipidemic diet
Carbohydrates as starch	80 %	72.75 %
Protein as casein	10 %	10 %
Fats as corn oil	5 %	5 %
Salt mixture	4 %	4 %
Vitamins mixture	1 %	1 %
Cholesterol	0 %	2 %
Bile salts	0 %	0.25 %
Sheep tail fat	0 %	5 %

2.4.4. Blood sampling and analysis

Blood samples were collected after six weeks in tubes contain heparin as an anticoagulant from the eye plexuses under diethyl ether anesthesia and then centrifuged at 3000 rpm for 20 min. to obtain plasma, which was kept frozen until analysis. The total cholesterol was analyzed calorimetrically according to **Richmond**, **(1973)** method**.** HDL - cholesterol was determined according to **Lopez et al. (1977)** method**.** Acording to **kikuchi et al. (1998)** LDL – cholesterol was calculated which LDL cholesterol = total cholesterol – (HDL cholesterol + triglyceride /5). Risk ratio was calculated according the formula of **Lopez et al. (1977)**

which Risk ratio = total cholesterol /HDL cholesterol . Atherogenic Index (AI) was calculated according to **Lee and Niemann (1996)** using following equation:

Atherogenic Index (AI) = Total cholesterol - HDL cholesterol / HDL cholesterol

The triglycerides were analyzed according to **Fossati and Prencipe (1982)** method. Alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) activities were measured according to the method described by **Reitman and Frankel (1957).** Total protein was determined according to **Weichselbaum (1946)** method ., and albumin was determined according to **Doumas et al. (1971)** method.

2.4.5. Histopathology

Liver from the experimental groups were immediately fixed in 10% formalin, then treated with conventional grades of alcohol and xylol, embedded in paraffin and sectioned at 4–6 lm thickness. The sections were stained with Hematoxylin and Eosin (H&E) stain for studying the histopathological changes **(Lillie, 1965).**

2.5. Statistical analysis

The results of the animal experiments were expressed as the Mean ± SE and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p<0.05$ was used as the criterion of statistical significance.

3. RESULTS AND DISCUSSION

The degree of N-deacetylation of the chitosan was determined by titration as described in the present materials and methods. The final deacetylation degree was 85%. Various absorption bands within the 4000-400 cm^{-1} range were recorded in the FTIR spectra of chitosan. Different stretching vibration bands were observed in the range 3425-2881 cm^{-1} related to ν(N-H) in ν(NH_2) assoc. in primary amines . The band at 3425-3422 cm^{-1} could be assigned to ν(N-H), ν(O-H) and ν(NH_2) which present in chitosan in different amounts among which NH_2 groups being the least. The presence of methyl group in $NHCOCH_3$, methylene group in CH_2OH and methylene group in pyranose ring was proved by the corresponding stretching vibrations of these groups in the range 2921-2879 cm^{-1} (Guo et al. 2005). A peak at 1156 cm^{-1} was assigned to the structure of saccharide. The peak at 1354 cm^{-1} was assigned to CH_3 in amide group. The broad peak at 1021 and 1098 cm^{-1} indicated the C-O stretching vibration in chitosan and peaks at 1628 and 1540 cm^{-1} were due to -C=O stretching (amide I) and NH stretching (amide II). The absorption bands at 1151 cm^{-1} was assigned to the anti-symmetric stretching of C-O-C bridge, and 1098 and 1021 cm^{-1} were assigned to the skeletal vibrations involving the C-O stretching. In none of this spectra was there any sharp absorptions at *circa* 3500 cm^{-1}, which confirms that the hydroxyl groups in positions C2 and C6 of the chitosans are involved in intra- and intermolecular hydrogen bonds.

The XRD pattern of chitosan prepared from shrimp shells waste illustrates two characteristic broad diffraction peaks at (2θ) = 10º and 20º that are typical fingerprints of semicrystalline chitosan as shown in Fig. 3 **(Bangyekan et al. 2006)**. The peaks around 2θ = 10º and 2θ = 20º are related to crystal I and crystal II in chitosan structure **(Ebru et al. 2007; Marguerite 2006)** and both these peaks attributes a high degree of crystallinity to the prepared chitosan **(Julkapli and Akil 2007)** as shown in Figure 3.

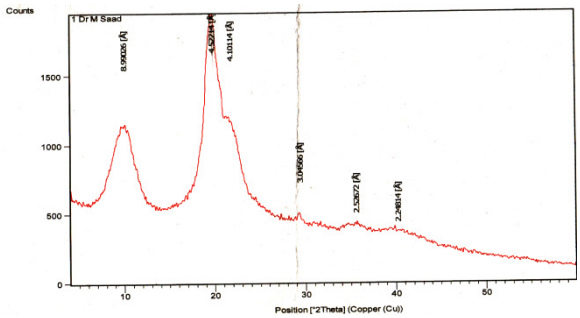

Figure - 3: shows X – Ray Diffraction of chitosan.

Hyperlipidemia is a major contributor for health problems worldwide and leads especially to atherosclerosis, resulting in coronary heart diseases (CHD). According to WHO by 2020, 60% of the cardiovascular cases will be of Indian origin **(Sethupathy et al. 2002)**. Hyperlipidemia induces the damages in various tissues, which in turn, alters the cellular functions leading to cell damage and many pathological conditions **(Chander et. al. 2003)**. A high-fat diet may cause elevated levels of cholesterol, which ultimately leads to obesity. Elevated cholesterol level particularly LDL, VLDL increases the risk of cardiovascular diseases particularly coronary heart disease (CHD)**(Aparna 2003)**. Increase in HDL cholesterol reduces the risk of CHD **(Martin and Annie 1998 and Takaaki et.al. 2001).** Reduction of 1% cholesterol can lead to 2-3% reduction of CHD risk **(Ornish and Rosner 2005)** .The high fat diet (HFD) administered in present study for effective hyperlipidemia. induction. The significant ($p<0.05$) change in lipid profile noticed in the experimental animals confirmed the induction of hyperlipidemia in HFD fed rats (Tables 2 and 3). High fat diet increased

Table - 2: Ttotal cholesterol , triglycerides and HDL-C (mg/dl) in rats fed hyperlipidemia induced diets with different levels of chitosan in drinking water.

Group / Parameter	Total cholesterol mg/dl	Triglyceride mg/dl	HDL-C mg/dl
Negative control	72.9±1.9 [a]	80.98±2.49 [a]	40.48±1.73 [a]
Positive control	135.43±4.16 [b]	127.14±3.43 [b]	21.26±0.69 [b]
Chitosan 300 mg/L	97.93±2.04 [c]	105.77±4.07 [c]	29.91±1.56 [c]
Chitosan 450mg/L	86.11±2.16 [d]	91.24±1.87 [d]	36.78±0.85 [d]

(a,b,c,d) Means in the same column followed by the same letters do not differ significantly , and means followed by different letters differ significantly at (P<0.05)

Table - 3: LDL-C mg/dl , risk ratio and atherogenic index AI , in rats fed hyperlipidemia induced diets with different levels of chitosan in drinking water.

Group / Parameter	LDL-C mg/dl	Risk ratio	Atherogenic index
Negative control	16.23±0.86 [a]	1.8±0.82 [a]	0.8±0.87 [a]
Positive control	89.75±0.62 [b]	6.42±0.34 [b]	5.38±0.35 [b]
Chitosan 300 mg/L	46.87±1.02 [c]	3.27±0.31 [c]	2.28±0.24 [c]
Chitosan 450mg/L	31.08±0.78 [d]	2.34±0.29 [d]	1.34±0.08 [d]

(a,b,c,d) Means in the same column followed by the same letters do not differ significantly , and means followed by different letters differ significantly at (P<0.05)

triglycerides level and leads to hardening of arteries **(Guido and Joesph 1992 and Joris et al. 1983)**. The present study showed that HFD significantly (p<0.05) increased TG level when compared with standard pellet treated rats. Treatment with chitosan at the different dose levels (300 and 450 mg/L of drinking water) for 6 weeks showed significant (p<0.05) decrease in triglyceride and total cholesterol levels in hyperlipidemic rats compared to positive control. HDL is a beneficial lipoprotein synthesized in intestine and liver which protects the system from the pathogenesis of atherosclerosis **(Xu et. al. 2005)**. In the present study, it is noticed that HDL cholesterol level in plasma increased significantly (p<0.05) in chitosan treated hyperlipidemic rats.(29.91 and 36.78 mg/dl for 300 and 450 mg/L chitosan in water respectively compared with positive control 21.26 mg/dl.

Increase in LDL level causes deposition of cholesterol in the arteries and aorta and hence is a leads to CHD. LDL transports cholesterol from the liver to the periphery **(Boden and Pearson2000, and Pedersen 2001)**. The fortification of LDL from oxidation and decrease in oxidative stress might therefore be useful for prevention of atherosclerosis associated CVD. In the present study administration of chitosan at two different dose levels effectively reduced LDL cholesterol content of hyperlipidemic rats. For a good lipid lowering therapy, a drug should be able to significantly lower LDL and increase HDL cholesterol concentration and this appreciably decreases the fatty cytoplasmic vaculated cells in liver parenchyma and prevents hepatic necrosis and this correlates with the present study **(Steinberg and Gotto 1999)**. Reduced LDL and increased HDL concentration were observed in the present study, thereby suggesting that this formulation could be used as a good lipid lowering therapeutic agent. Atherogenic index (AI) signifies the deposition as foam cells, plaque or fatty infiltration in circulatory system. An increased atherogenic index indicates high risk of susceptibility of heart and kidney to oxidative damage **(Kesavulu et al. 2001)**.

In the present study, treatment with chitosan at the dose of 300 and 450 mg/L drinking water indicated significant (p<0.05) decrease in atherogenic index compared with positive control , thus indicating the protective role of test formulation against atherogenesis. This results can be explained due to strong positive charge carried by chitosan molecule (amino groups) causes it to bind negatively charged substrates such as lipids, while chitosan binds fat in the intestine, blocking absorption, and has been shown to lower blood cholesterol in animals and humans. (**Ormrod et al., 1998 and Gallaher et al., 2000**). In addition the reduction of endogenetic cholesterol because of the interruption of enterohepatic bile acid circulation will influence cholesterol metabolism (**Razdan and Pettersson 1996)** . Behind 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, which is at neutral pH, chitosan forms a

Table - 4: Plasma AST and ALT activities , total protein mg/dl and albumin mg/l in rats fed hyperlipidemia induced diets with different levels of chitosan in drinking water.

Group / Parameter	AST (U/L)	ALT (U/L)	Total protein	Albumin
Negative control	63.48±0.58 [a]	24.7±1.84 [a]	6.28±0.21 [a]	3.03±0.28 [a]
Positive control	74.19±1.33 [b]	39.98±1.39 [b]	6.49±0.45 [a]	2.82±0.77 [a]
Chitosan 300 mg/L	71.07±0.84 [c]	35.14±1.39 [c]	6.11±0.35 [a]	2.96±0.3 [a]
Chitosan 450mg/L	64.39±0.83 [a]	31.21±1.05 [d]	6.09±0.73 [a]	2.74±0.33 [a]

(a,b,c,d) Means in the same column followed by the same letters do not differ significantly , and means followed by different letters differ significantly at (P<0.05)

precipitate and prevents the digestion of fat (**Zhou et al., 2006**). So the actions of chitosan include interference of lymphatic absorption of cholesterol and fat increased fecal excretion of neutral steroids and fat, and improvement of liver function (**LeHoux and Grondin, 1993 and Zhang et al., 2008**).

Table 4 presents the results of plasma AST and ALT activities in the controls and experimental groups. There were significant increases ($P < 0.05$) in the plasma AST, and ALT activities of hyperlipidemic rats as compared to normal control rats . The present finding are in agreement with those obtained by **(Ahmed et al. 1987)** who found that hypercholesterolemia state significantly stimulate ALT and AST activity in the plasma. Significant decrease ($P < 0.05$) in plasma AST and ALT activity of rats fed hyperlipidemia-induced diet which drink water containing chitosan, at doses 300 and 450 mg/L compared to hyperlipidemia control . In the study, it was observed that as a result of hyperlipidemia, enzymes such as AST and ALT were released into blood. Their increase in the plasma activities of these enzymes was directly proportional to the degree of cellular damage. These values decreased by chitosan.

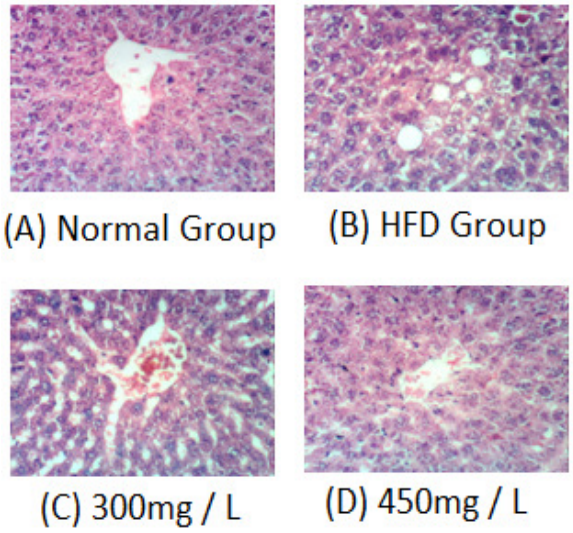

Figure - 4: Histopathological changes detected in the liver of (A) normal group, (B) HFD group, (C) Chitosan at 300mg/L and (D) Chitosan at 450 mg/L.

No changes in total protein and albumin content were observed by chitosan groups when compared with hyperlipidemia control group .

Figure 4 presents the histopathological observation of liver revealed the accumulation of triglycerides and fatty changes. Groups (A and D) showing no histopathological changes ,while group (B) high fat diet showing fatty change of hepatocytes and group (c) showing small focal hepatic necrosis associated with inflammatory cells infiltration. The administration of chitosan at 450 mg/L drinking water reversed the pathological changes and brought back the normalarchitecture of the liver.

To sum up, the effect of chitosan was studied in experimental rats, where hyperlipidemia was induced through high fat diet. The administration of chitosan to the hyperlipidemic rats significantly reduced total cholesterol, TG, and LDL .The chitosan revealed maximum protective effect at a dose of 450 mg/L drinking water in comparison with 300 mg/L. Further in-depth studies can result in the development of an effective chitosan as anti-obesity drug.

4. CONCLUSION

Degree of deacetylation of chitosan determined by titration , it was 85% , chitosan sample was characterized by FTIR and X-ray diffraction. Various absorption bands within the 4000-400 cm^{-1} range were recorded in the FTIR spectra of chitosan and in X-ray diffraction the prepared chitosan showed a high degree of crystallinity, the results showed that chitosan could decrease levels of triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and increase (HDL-C). In conclusion, research findings of this study demonstrated the potential of plasma lipids-lowering effect of chitosan in rats.

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5. REFERENCES

Ahmed FA, El-Desoky GE, El-Saadawy SS and Ramadan ME. (1987). Carbohydrates and lipids changes in rats administrated certain synthetic and natural food colors. **Minia J. Agric. Res. Dev.,** 9(3): 1101-1116.

Aparna Berteri R (2003). **Risk of coronary artery heart disease, Health Screen**; 1:28-29.

Bangyekan C, Aht-Ong D and Srikulkit K (2006). Preparation and properties evaluation of chitosan-coated cassava starch films. **Carbohyd Polym**., 63(1):61–71

Boden WE, Pearson TA (2000). Raising low levels of high-density lipoprotein cholesterol is an important target of therapy. **Am J Cardiol.,** 85:645-650.

Brown MS and Goldstein JL (1986). A receptor-mediated pathway for cholesterol homeostasis. **Science.,** 232:34-47.

Campbell JA (1961). Methodology of protein evaluation. **RAG Nutrition Document R.** 101 add. 37, June meeting , New York.

Chander R, Kapoorn K and Singh C (2003). Lipid per oxidation of hyperlipidemic rat serum in chronic ethanol and acetaldehyde admimistration. **J Biosciences,** 13:289-274.

Davidson MH, Kurlandsky SB, Kleinpell RM and Maki KC (2003). Lipid management and the elderly. **Prev Cardiol,** 6:128 - 33.

Deuchi K, Kanauchi O, Imasato Y and Kobayashi E. (1995). Effect of the viscosity or deacetylation degree of chitosan on fecal fat excreted from rats fed on a high-fat diet. **Bioscience Biotechnology and Biochemistry,** 59(5), 781–785.

Doumas BT, Watson WA and Biggs HG (1971). Albumin standards and measurement of serum albumin with bromcresol green. **Clin Chim Acta.,** 31: 87–96.

Ebihara K and Schneeman BO (1989). Interaction of bile acids, phospholipids, cholesterol and triglyceride with dietary fibers in the small intestine of rats. **J. Nutr.,** 119, 1100–1106.

Ebru G, Dilay P, Cuney H, Unlu OA, Nurfer G (2007). Synthesis and characterization of chtiosan—MMT bio composite systems. **Carbohyd Polym** 67:358**.**

Fossati P and Prencipe L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. **Clinic. Chem.** 28: 2077–2080.

Gallaher CM, Munion J, Hesslink RJR, Wise J and Gallaher DD (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.

Guido S and Joesph T (1992). Effect of Chemically different antagonists on lipid profile in rat fed on a high fat diet. **Indian J Exp Biol.,** 30: 292-294.

Guo M, Diao P, Cai S (2005). Hydrothermal growth of well aligned ZnO nanorodarrays: dependence of morphology and alignment ordering upon preparing conditions. **J Solid State Chem.,** 178(6): 1864–1873.

Joris I, Zand T, Nunnari JJ, Krolikowski FJ and Majno G. (1983). Studies on the pathogenesis of atherosclerosis. I. Adhesion and emigration of mononuclear cells in the aorta of hypercholesterolemic rats. **Am J Pathol**., 113:341-58.

Julkapli MN, Akil MH (2007). X-ray powder diffraction (XRD) studies on kenaf dust filled chitosan bio-composites. **AIP Conf Proc.,** 989:11

Varady KA and Jones PJH (2005). Combination diet and exercise interventions for the treatment of dyslipidemia: an effective preliminary strategy to lower cholesterol levels?. **Journal of Nutrition,** 135,(8. 1829–1835.

Kanauchi O, Deuchi K, Imasato Y, Shizukuishi M and Kobayashi E (1995). Mechanism for the inhibition of fat digestion by chitosan and for the synergistic effect of ascorbate. **Bioscience Biotechnology and Biochemistry,** 59, 5, 786–790.

Kesavulu M, Kameswara M, Rao B and Giri R. (2001). Lipid peroxidation and antioxidant enzyme status in type to diabetics with coronary heart disease, **Diabetes Res Clin Pract.,** 53: 33-39.

Kikuchi HH, Onodera N, Matsubara S, Yasuda E, Chonan O, Takahashi R and Ishikawa F. (1998**).** Effect of soy milk and bifidobacterium fermented soy milk on lipid metabolism in aged ovariectomized rats. **Bioscience, Biotechnology and Biochemistry.** 62, 9**,** 1688 – 1692.

Kovanen PT, Brown MS, Basu SK, Bilheimer DW and Goldstein JL (1981). Saturation and suppression of hepatic lipoprotein receptors: a mechanism for the hypercholesterolemia of cholesterol-fed rabbits. **Proc Natl Acad Sci.,** 78: 1396-1400.

Lee R and Niemann D. (1996). **Nutritional Assessment 2nd ed Mosby Missou USA**.

LeHoux JG and Grondin F 1993. Some effects of chitosan on liver function in the rat. **Endocrinol.,** 132, 1078-1084.

LeHoux JG and Grondin F (1993). Some effects of chitosan on liver function in the rat. **Endocrinology,** 132(3), 1078–1084.

Lillie RD (1965). **Histopathological technique and practical histochemistry.** Third ed. Published by the Blakistar Division of McGraw-Hill Book Co., New York, Toronto, London.

Lopez MF, Stone S, Ellis S and Collwell JA (1977). Cholesterol determination in high density lipoproteins separated by three different methods. **Clin. Chem.,** 23: 882 – 886.

Maezaki Y, Tsuji K, Nakagawa Y, Kawai Y, Akimoto, M and Tsugita, T. (1993). Hypocholesterolemic effect of chitosan in adult males. **Bioscience Biotechnology and Biochemistry,** 57(9), 1439–1444.

Marguerite R (2006). Chitin and chitosan: properties and applications. **Prog Polym Sci**., 31(7):603–632**.**

Martin M, Annie B. (1998). The positive relationship between alcohol and heart disease in Eastern Europe: potential physiological mechanisms. **J R Soc Med.,** 9:402-407.

Muzzarelli, RA, Ilari, P and Petrarulo M (1994). Solubility and structure of N-carboxymethylchitosan. **Int. J. Biol. Macromol.,** 16, 177–180.

Muzzarelli RAA. (1996). **Chitosan-based dietary foods. Carbohydrate Polymers,** 29, 309–316.

Nakamura H, Izumiyama N, Nakamura K and Ohtsubo K. (1989). Age-associated ultra structural changes in the aortic intima of rats with diet induced hypercholesterolemia. **Atherosclerosis.** 79 (2-3): 101 – 111.

Ormrod DJ, Holmes CC and Miller TE 1998. Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. **Atherosclerosis,** 138: 329-334.

Ornish D, Rosner B. (2005). The effect of intake of dietary fat **JAMA,** 49:263-267.

Pedersen TR (2001). Low density lipoprotein cholesterol lowering is and will be the key to the future of lipid management. **Am J Cardiol,** 87:8B-12B.

Razdan A and Pettersson D (1996). Hypolipidaemic, gastrointestinal and related responses of broiler chickens to chitosans of different viscosity. **Br. J. Nutr.,** 76(3): 387-397.

Razdan A, Pettersson D and Pettersson J (1997). Broiler chicken body weights, feed intakes, plasma lipid and small-intestinal bile acid concentrations in response to feeding of chitosan and pectin. **Br J Nutr.,** 78: 283-291.

Reitman S and Frankel S (1957). Colorimetric determination of GOT and GPT**.** **Am.J.Clin.Path.,** 28:56 - 60.

Richmond W (1973). Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. **Clinic. Chem.,** 19:1350–1356.

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

Sethupathy S, Elanchezhiyan C, Vasudevan K and Rajagopal G (2002). Antiatherogenic effect of taurine in high fat diet fed rats. **Indian J Exp Biol.,** 40:1169-1172.

Steinberg D, Gotto AM Jr (1999). Preventing coronary artery disease bylowering cholesterol levels- Fifty years from bench to bedside. **JAMA,** 282: 2043-2050.

Sugano M, Fujikawa T, Hiratsuji Y, Nakashima K, Fukuda N and Hasegawa Y (1980). A novel use of chitosan as a hypocholesterolemic agent in rats. **Am. J. Clin. Nutr.,** 33, 787–793.

Sugano M, Watanabe S, Kishi A, Izume M and Ohtakara A (1988). Hypocholesterolemic action of chitosans with different viscosity in rats. **Lipids,** 23(3), 187–191.

Takaaki S, Vaijinth SK and Moti LK (2001). Niacin, but not gemfibrozil, selectively increases LP-AI, a cardioprotective subfraction of HDL, in patients with low HDL cholesterol. **Arterioscler Thromb Vasc Biol.,** 21:1783-1789.

Vahouny GV, Satchithanandam S, Cassidy MM, Lightfoot FB and Furda I. (1983). Comparative effects of chitosan and cholestyramine on lymphatic absorption of lipids in the rats. **The American Journal of Clinical Nutrition,** 38, 278–284.

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

Weichselbaum TE (1946). Quantitative colorimetric determination of total protein in serum. **Am. J. Clin. Pathol.,** 7:40.

Xu Y, He Z and King GL. (2005) Introduction of hyperglycemia and dyslipidemia in the pathogenesis of diabetic vascular complications. **Curr Diab Rep,** 5:91-97.

Ylitalo R, Lehtinen S, Wuolijoki E, Ylitalo P and Lehtimaki T (2002): Cholesterol-lowering properties and safety of chitosan. **Arzneimittel-Forschung-Drug Res.,** 52: 1-7.

Youn DK., No HK and Prinyawiwatkul W. (2007). Physical characteristics of decolorized chitosan as affected by sun drying during chitosan preparation. **Carbohydrate Polymers,** 69, 707–712.

Zhou K, Xia W, Zhang C and Yu L (2006). In vitro binding of bile acids and triglycerides by selected chitosan preparations and their physicochemical properties. **LWT-Food Sci. Technol.,** 39: 1087-1092.