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Effect of dietary chitosan extracted from exoskeleton of freshwater crab *Sartoriana spinigera* on relative liver weight and lipid content of liver in albino rats

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Abstract : Nonalcoholic fatty liver disease (NAFLD) is an important complication of the metabolic syndrome, which is becoming an increasingly common cause of chronic liver disease. Nowadays, remedies of natural origin are in demand for cure of severe liver and heart related problems. Chitosan, a biopolysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine, has been shown to have properties of decreasing lipid content in the body, including liver. The present study was carried out to investigate the efficacy of chitosan extracted from carapace of freshwater crab *Sartoriana spinigera* on lowering of lipid content in liver and maintaining the normal liver weight of hyperlipidemia induced albino rats. 20 albino rats were randomly divided into 4 groups fed with different experimental diets: Group A-Basal diet, Group B-High fat diet, Group C-high fat diet+5% chitosan and Group D-High fat diet +5% hypolipidemic drug Ezetimibe. The experiment was conducted for 30 days after which rats were sacrificed and their liver were obtained. Wet weight of liver of rats was found to be 4.640±.0021 g, 5.723±.0017 g, 4.670±.0016 g, 4.670±.0001 g in group A,B,C and D respectively. Lipid content in liver of rats of all groups was also measured and was found to be 0.062±.0004 g, 0.161±.0017 g, 0.094±.0010 g and 0 .115±.0004 g in group A, B, C and D respectively. Statistical analysis by Student's t-test revealed that rats of group C that was fed with chitosan had lower liver weight than that of group B fed with high fat diet at 0.1% significance level. Lipid content in liver of group C rats was also found to be significantly lower than lipid content of liver of group B at 0.1% significance level indicating that chitosan extracted from *Sartoriana spinigera* can prevent the risk of NAFLD by reducing lipid content in the liver.

Keywords : : NAFLD, Chitosan, *Sartoriana spinigera*, liver weight, lipid content, Student's t-test

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of abnormal liver dysfunction leading to a wide spectrum of liver disease, ranging from fatty liver alone to steatohepatitis. Intake of high concentration of cholesterol and fatty food directly and indirectly are responsible for NAFLD. The overall prevalence of NAFLD is estimated to be 20-30% in the general population (Kim *et al*., 2012)[1], and it has become the most common cause of chronic liver disease and liver transplantation in western countries (Nakao *et al*., 2006)[2]. High deposition of lipid in the liver followed by increase in the weight of liver are the basic symptoms that indicate towards NAFLD. The treatments for NAFLD currently focuses on decreasing metabolic risk factors, with therapy mainly targeting lifestyle adaptations such as gradual weight loss by diet and exercise (Mattar *et al*., 2005; Rafiq and Younossi, 2008)[3,4]. But, no convincing effect has been shown to improve the liver conditions especially liver weight.

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Presently, society is demanding for treatments that are of natural origins. Therefore, Zootherapy has gained keen interest in the field of medicine. One such zootherapeutic medicine is Chitosan. Chitosan is a linear polysaccharide biopolymer which is a partial deacetylated form of chitin and is found in exoskeleton of crustaceans, arthropods and cell wall of fungi. It is composed of 80% deacetylated D-glucosamine and 20% acetylated N-acetyl-D-glucosamine. Chitosan is known to be a dietary fibre and is a cationic polysaccharide which has an affinity towards anionic fatty acid chains, thus binding with them and not letting them get deposited in the body.

Chitosan has been extracted mostly from exoskeleton of marine invertebrates. Very less research has been done on chitosan extracted from freshwater crabs. *Sartoriana spinigera* which is a freshwater crab found abundantly in the freshwater bodies of Jharkhand and is consumed not only as a local delicacy but is also of ethnomedicinal significance as it is used by tribal's of Jharkhand against many health problems such as microbial infections, gastrointestinal disorders, arthritis.

The aim of this study was to determine if Dietary chitosan extracted from freshwater crab *Sartoriana spinigera* could reduce the lipid content in liver and liver weight of hypercholesterolemia induced albino rats.

MATERIALS & METHODS

Extraction of Chitosan: The method of Takiguchi (1991)[6] was selected from other methods for extraction of chitosan using minimum chemicals. Chitosan was deacetylated form of chitin. The 3 processes that involved extraction of chitosan were demineralization, deproteinization and deacetylation.

Experimental Design-

Animals and diet: 20 albino rats, each measuring about 80 grams were purchased from local rearing laboratory. Rats were divided into 4 groups (A, B, C and D) and housed in metallic cages under healthy condition. Rats were maintained at room temperature. There was 12 hours daytime light between 6AM to 6PM and 12 hours of dark. Water and basal diet were provided ad-libitum for 3 days as acclimatization period. Rats were fed with respective diets at 9 am and 9 pm. Experimental diets were given for 30 days.

4 groups of rats were fed with following experimental diets following Kumar *et al* (2009)[7].

Group A-basal diet

Group B-high fat diet

Group C-high fat diet + 5%chitosan

Group D-high fat diet+ 5% hypolipidemic drug (ezetimibe)

Table 1: Composition of experimental diet

Component	GROUP A Basal diet g/1000g	GROUP B High fat diet g/1000g	GROUP C High fat diet + chitosan g/1000g	GROUP D High fat diet + Ezetimibe g/1000g
Casein	215	147	147	147
Gram+wheat+maize	450	306.6	306.6	306.6
Sucrose	200	140	140	140
Soyabean oil	50	15	15	15
Vitamin+mineral mixture	35	35	35	35
Cellulose	50	50	-	-
Chitosan	-	-	50	-
Hypolipidemic drug	-	-	-	50
Cholesterol	-	5	5	5
Deoxycholic acid	-	2	2	2
Coconut oil	-	300	300	300

Analytical method

After 30 days, rats of all groups were sacrificed and livers were collected and weighed. After weighing, liver was dried and lipid content of liver was estimated by Folch method (Folch *et al,* 1957)[5].

Statistical analysis

The variability of the results was expressed as mean ± standard deviation. The significance of the differences between mean values of different groups was determined using Student's t test.

RESULT & DISCUSSION

Table 2: Effect of different experimental diets on various parameters of liver of albino rats

	Weight of Wet liver (g)	Weight of Dry liver (g)	Total lipid content of liver (g)
GROUP A	4.640±.0021***	1.03±.0031***	.062±.0004***
GROUP B	5.723±.0017	1.32±.0016	.161±.0017

*= significant at 0.1%

	Weight of wet liver (g)	Weight of dry liver (g)	Total lipid content of liver (g)
GROUP B	5.723±.0017	1.32±.0016	.161±.0017
GROUP C	4.670±.0016***	1.00±.0010***	.094±.0010***

*= significant at 0.1%

	Weight of wet liver (g)	Weight of dry liver (g)	Total lipid content of liver (g)
GROUP C	4.670±.0016	1.00±.0010	.094±.0010***
GROUP D	4.670±.0001	.92±.0025***	.115±.0004

*= significant at 0.1%

Table 2 showed various parameters of weight of liver and lipid content of liver of treated rats.

Group A fed with basal diet showed least weight of wet liver i.e., 4.640±.0021 g/rat. Group C and D showed lesser wet weight of liver i.e., 4.670±.0016 g/rat and 4.670±.0001 g/rat respectively. Group B fed with high fat diet showed highest weight of wet liver i.e., 5.723±.0017 g/rat. Statistical analysis between group B and C showed that group C had lower wet weight of liver than group B at 0.1% significance level. Statistical analysis between group C and D also showed that group C had lower wet weight of liver than group D at 0.1% level of significance.

But dry weight of liver was found to be lowest in group D treated with hypolipidemic drug. Group A fed with basal diet and group C with chitosan were able to show similar results for dry weight of liver i.e., 1.03±.0031 g/rat and 1.00±.0010 g/rat respectively. Group B fed with high fat diet showed highest dry weight of liver i.e., 1.32±.0016 g/rat. Statistical analysis between group B and C showed that group C fed with chitosan had lower dry weight of liver than group B at 0.1% significance level. Similarly, statistical analysis between group C and D also showed that group C had lower dry weight of liver than group D at 0.1% significance level.

Total lipid content in liver was found to be lowest in group A i.e., 0.062±.0004 g and highest in group B fed with high fat diet i.e., 0.0161±.0017 g. Group C fed with high fat diet and chitosan also showed lower fat content of liver i.e., 0.094±.0010 g, which was found to be lower than fat content of liver of group D i.e., 0.115±.0004 g.

Statistical analysis between group B and C showed that group C had lower fat content in liver than group B at 0.1% significance indicating that chitosan does not allows deposition of lipid in liver. Statistical analysis between group C and D also showed that group C had lower fat content in liver than group D indicating that chitosan is a potent agent in decreasing fat content than commonly used hypolipidemic drug.

Present study revealed that chitosan extracted from exoskeleton of *Sartoriana spinigera* is capable of preventing NAFLD when taken in diet and reduce the content of lipid in liver. Liver weight of rats also decreased in 30 days when fed with chitosan. Similar results were observed by Hanaa *et al* (2013)[8], according to which liver

weight decreased by 60% in animals that received 500mg low molecular weight chitosan oligosaccharide II/ kg for 14 consecutive days after an oral dose of 25 µg TCDD /kg body weight. Relative liver weight increased due to fatty vacuolization in rats gavaged with 10 or 30 µg TCDD/kg, respectively for 1 week. Also, feeding 3.6 g low molecular weight chitosan /kg diet caused a significant decrease in liver relative weight at (pd" 0.01), when compared with liver of rats fed with TCDD(2,3,7,8 tetrachlorodibenzo-p-dioxin). B. Lamiaa (2011) reported that dietary 5% chitosan to adult rats for 6 weeks caused a significant decrease in body weight and liver weights. However, no significance difference was seen in liver weights among the test groups in experiment done by Se-Jae Kim *et al* (1998)[9].

Hanaa *et al* (2013)[8] reported that feeding 3.6 g low molecular weight chitosan/kg diet caused a significant decrease in body weight at (pd" 0.01) when compared with body weight of rats fed with TCDD.

CONCLUSION

Abnormal liver weight and accumulation of high content of lipid in the liver are the primary symptoms of NAFLD. Remedies are required to cure NAFLD such that more severe problems of chronic liver disease can be prevented. In this experiment, Chitosan extracted from freshwater crab *Sartoriana spinigera* has proved to be a natural remedy. Chitosan can maintain the liver weight at normal level despite of intake of cholesterol. Chitosan can also reduce the lipid content in liver, thereby decreasing the risk of NAFLD. Use of chitosan from a freshwater crab will not only acknowledge the society about its importance but will also enhance its economic importance and will be an encouragement for the farmers to culture *Sartoriana spinigera*.

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