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Biochemical alterations of Chitosan on Liver function tests and Immunoglobulin in Experimental induced Non Alcoholic Fatty Liver Disease in Rats

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ABSTRACT

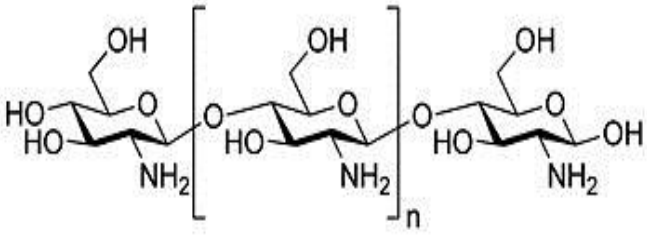
The main objective of this study to investigate the alterations of chitosan on Hepatic function tests and Immunoglobulin in Experimental induced Nonalcoholic fatty liver disease [NAFLD] in rats. Thirty male albino rats were divided into three groups [10 rats each]. The first group fed a normal diet and represents the control group. The second group [NAFLD] fed normal diet enriched with1% cholesterol and 2% coconut oil and act as positive control [+ve control]. The third group fed on normal diet enriched with1% cholesterol and 2% coconut oil and chitosan to the basal diet at a dose 5%. Samples collected after 2,4and6 weeks after induction from treatment .serum was used for estimation of Liver Function Tests as Serum Aspartate aminotransferase [AST], Alanine aminotransferase [ALT], Gammaglutamyltransferase [GGT] and Immunoglobulins as Serum ImmunoglobulinA [IgA], Immunoglobulin G [IgG]. Our Results revealed a significantly increase in serum AST, ALT, GGT, Serum IgA, IgG were significantly increase was observed. The behavioral biochemical results indicated treatment with chitosan showed significant changes and improves these parameters.

Keywords: NAFLD, Chitosan, Liver Function Tests, *Immunoglobulins*.

1. INTRODUCTION

Nonalcoholic fatty liver disease [NAFLD] represents a wide spectrum of disorders, the hallmark of which is hepatic steatosis [1]. Liver enzyme as Alanine aminotransferase, [AST] is found in serum and in various bodily tissues, but is most commonly associated with the liver. It is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health. Aspartate aminotransferase is similar to alanine transaminase [ALT] in that both enzymes are associated with Liver parenchymal cells [2]. Chitosan is a linear polysaccharide composed of randomly distributed β-[1-4]inked D-glucosamine [deacetylated unit] and N-acetyl-D-glucosamine [acetylated unit]. It is made by treating shrimp and other crustacean shells with the alkali sodium hydroxide [3].

Structures of chitin and chitosan: Poliglusam; Deacetylchitin; Poly-[D]lucosam [4].



2. MATERIALS AND METHODS

2.1Animals and chemicals:

Male white albino rats, 6-8weeks age and weighting[150– 180g] were used in the experiment. Rats were housed in separate metal cage with free access to water. Rats were kept under constant and nutritional environmental condition throughout the experiment. Rats were left for15 days before beginning of experiment for acclimatization. Cholesterol and coconut oil were purchased from El-Goumhouria Co. for Trading Chemicals, Egypt. Hepatic lipid metabolism induced NAFLD by continuous supplementation of high fat diet [HFD] was prepared by High Cholesterol[1% wt/wt] and [Coconut oil 2%wt/wt] to normal ration according to [5].

2.2 Chitosan:

Powder from crab shells poly-[1-4-βDglucopyranosamine], 2-Amino-2-deoxy βDgluco pyranone [3]. Chitosan to the basal diet at a dose 5% according to [6].

2.2.1 Preparation and dosage of chitosan:

Chitosan was prepared by deacetylation of chitin present in the shell of shrimp as described by [7]. The prepared chitosan to the basal diet at dose 5% [1kgdiet\50gmchitosan] according to [6].

2.3 Experimental design:

Rats were divided into 3 groups [10 per each] main groups placed in individual cages and classified as follows:

Group I was fed on normal diet and served as control group.

Group II was fed on high fat diet [Normal NAFLD] for 12 weeks.

Group III was fed on high fat diet [NAFLD] and treated chitosan at a dose of 5% [1kgdiet\50gmchitosan] according to [6].

2.4 Sampling:

After overnight fasting blood samples was collected from all animal groups [control and experimental groups] after12 weeks for detection of NAFLD [hyperlipidemia]. Then samples were collected after 2, 4 and 6 weeks from onset of treatment.

2.4.1Blood samples:

Blood Samples were collected from Medial Canthes of eye and collected in dry, clean and screw capped tubes then rats decapitated for liver tissue removal containing serum were separated by centrifugation at 2500 r.p.m for 15 minutes. The clean clear serum was separated by Pasteur pipette and kept in a deep freeze at -20C till used for determination of the biochemical Parameters: Serum liver function tests as serum AST[8], serum ALT[8], serum GGT[9], Immunoglobulin A Serum IgA[10], IgG [10].

2.5 Statistical analysis:

The obtained data were analyzed represented using the statistical package for social science [SPSS, 13.0 software, 2009[11], for obtaining mean and standard deviation and error. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping.

3. RESULTS AND DISCUSSION

The obtained results demonstrated in tables [1, 2 and 3] revealed that a significant increase in Serum AST, Serum ALT, Serum GGT after 2, 4 and 6 weeks compared with the normal control group These Results were nearly similar to [12] found that a significant increase in serum AST, ALT and GGT elevated liver enzyme may be due to fatty infiltrate [13].

On the other hand, Chavez-Tapia NC et al. (2012) [14] observed that a significant increase in serum alanine aminotransferase levels may be due to the NASH animal model involving choline-deficient diet fed rats it an increase in serum and portal alanine aminotransferase levels and hepatic TNF-α, IFNγ and TLR4. Higher TNF-α levels were detected in KCs and, most importantly, increased TNF-α,TLR4 expression, and macrophage /dendritic cell populations were found in ileal tissue specimens, demonstrating also the involvement of the gut in steatotic liver damage [15].

Table 1. Effect of chitosan on serum AST, ALT, GGT, serum IgA, IgG , After 2 weeks In Experimental induced Nonalcoholic fatty liver disease NAFLD in rats

Parameter/ group	AST U/L	ALT U/L	GGT U/L	IgA ng/ml	IgG ng/ml
Normal Control group	220.38^{bcd} ±6.37	$90.66e^{eh}$ ±4.86	55.52^{efg} ±5.09	2.59^{ef} ±0.20	29.68^{e} ±0.51
control NAFLD group	259.16^{ab} ±25.90	194.83^{a} ±14.85	65.68^{de} ±5.36	4.79^{c} ±0.05	80.37^{a} ±3.22
NAFLD Treated chitosan group	211.24^{bcd} ±15.15	184.10^{bc} ±3.45	62.55^{abc} ±0.46	2.36^{ef} ±0.28	20.10^{ef} ±2.09

Data are presented as (Mean ± S.E). S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at P< 0.05

Table 2. Effect of chitosan on serum AST, ALT, GGT, serum IgA, IgG after 4 weeks in Experimental induced Nonalcoholic fatty liver disease in rats

Parameter/ Group	AST U/L	ALT U/L	GGT U/L	IgA) ng/ml:	IgG ng/ml:
Normal Control group	162.42^{ef} ±4.00	105.06^{eh} ±2.21	47.80^{f} ±1.68	2.20^{de} ±0.12	32.76^{de} ±2.80
control NAFLD group	218.46^{bcd} ±20.24	167.84^{cde} ±13.82	55.54^{f} ±2.59	5.25^{b} ±0.09	43.22^{c} ±2.54
NAFLD Treated chitosan group	211.34^{abc} ±2.99	179.06^{bcd} ±1.69	50.29^{de} ±0.99	2.68^{de} ±0.15	26.29^{ef} ±0.86

Data are presented as (Mean ± S.E).S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at *P< 0.05*

Table 3. Effect of chitosan on serum AST, ALT, GGT, serum IgA, IgG tissue L-CAT L-SOD L-GSH after 6 weeks in Experimental induced Nonalcoholic fatty liver disease in rats

Parameter/ group		AST U/L	ALT U/L	GGT U/L	IgA ng/ml:	IgG ng/ml:
Normal group	Control	173.89^{def} ±7.81	115.02^{ehg} ±3.14	57.88^{ef} ±2.91	3.00^{e} ±0.15	25.00^{e} ±1.25
control group	NAFLD	236.88^{abc} ±11.86	199.37^{bc} ±13.67	69.31^{bc} ±1.73	6.23^{a} ±0.09	92.03^{ab} ±6.41
NAFLD chitosan	Treated group	229.99^{abc} ±7.71	166.98^{cde} ±6.26	38.44^{h} ±2.41	2.86^{e} ±0.17	27.37^{ef} ±0.51

Data are presented as (Mean ± S.E).S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at *P< 0.05*

Also Yanpallewar et al. (2002) [16] found that a significant increase in Serum AST, ALT, and γ-GT due to Liver cell destruction results in the leaking out of tissue contents into the blood stream. Serum AST, ALT, and γ-GT are the most sensitive markers employed in the diagnosis of liver diseases [17]. It seems also similar to [18] revealed that serum AST and ALT are the most sensitive markers employed in the diagnosis of hepatic damage due to their location in the cytoplasm and hence released into the circulation after cellular damage [19].

Moreover Radwan et al. (2008) [20] found a significant increase in AST and ALT activities is the drastic physiological effects directly caused by hepatotoxins interaction with cellular membranes, mitochondria or through action of free radicals. Hence, it may be related to extensive breakdown of liver parenchyma and renal tubules with subsequent enzyme release leading to their increase in blood [21].

However Raghavendran et al. (2004) [22] observed that a significant increase in serum ALT and AST may be due to Other enzymes that indicate parenchymal injury (when elevated) are ALT and AST. It is more likely that the rise in the AST is usually accompanied by an elevation in the activity of ALT, which plays a vital role in the conversion of amino acids to keto acids In addition to Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells [23]. Therefore, the marked release of AST and ALT from liver cytosol into circulation and an increase of γ-GT, a membrane enzyme, indicate severe damage to hepatic tissue membranes [24] Treatment with chitosan showed a significant decrease in Serum AST , serum ALT, Serum GGT concentration after 2,4and 6 weeks compared with control NAFLD group. These results were in accordance with [25]found that a significant decrease in serum ALT and γ-glutamyltranspeptidase [GGT] due to this effect of chitosan was associated with repressed serum levels of alanine aminotransferase [ALT], γ-glutamyl transpeptidase [GGT] [26].

The obtained results demonstrated in tables [1,2and3] revealed that A significant increase in serum IgA, IgG concentration after 2,4 and 6weeks compared with the normal control group. these results were similar to[27] showed that a significant increase in serum IgA, IgG may be due to the immune cell population, natural killer T[NKT] cells, which express NK cell markers and α/β T cell receptors, are reduced in steatotic, obese mice [28].

Moreover Brun et al. (2007) [29] found that a significant increase in serum IgA, IgG genetically obese mice have an impaired intestinal permeability leading to increased portal endotoxemia[30] . However [31] showed that a significant increase in serum IgA, IgG may be due to the adipose tissue has an important role in regulating energy utilization, vascular functions and immune system homeostasis. C-reactive protein [CRP],

interleukin[IL]-6, fibrinogen and plasminogen activator inhibitor-1 levels are higher in obese patients compared to healthy subjects have recently found that obese mice, after high fat and high cholesterol diets, express abnormal levels of macrophages and inflammation associated genes in adipose tissue and in liver[32].

These results also nearly similar to [33] found that a significant increase in serum IgA, IgG These findings indicate a crucial role for ox LDL in the fibrogenic process immunized mice on an HFC diet showed decreased foamy KCs compared with non immunized mice. This reduction in size is probably due to decreased plasma cholesterol levels, as the size of the foamy KCs is not always correlated with the inflammatory state of the liver [34].

Treatment with chitosan exhibited a significant decrease in serum IgA, IgG concentration in rats after 2,4and6 weeks compared with control NAFLD group. These results in accordance with[35] revealed that a significant decrease in serum IgA, IgG may be due to chitosan as a delivery system for administered vaccines. After administration of the chitosan-antigen vaccines it was generally found that the formulation induced significant serum IgG responses similar to and secretory IgA levels superior to what was induced by a parenteral administration of the vaccine.

Moreover Sevda Şenel et al. (2004) [36] found that chitosan including wound healing, bone regeneration, analgesic and antimicrobial effects potential application of chitosan to drug and vaccine delivery in veterinary species. Given the restrictions imposed by financial and animal restraint considerations, especially in farming applications, the veterinary drug delivery areas most likely to benefit from chitosan are the delivery of chemotherapeutics such as antibiotics, antiparasitics, anaesthetics, painkillers and growth promotants to mucosal epithelium for absorption for local or systemic activity, and the delivery of immunomodulatory agents to the mucosal associated lymphoid tissue for induction or modulation of local immune responses [37].

However Beong Ou Lim et al. (2004) [38] stated that the dietary fiber may have an immune regulatory effect on the intestinal immune system of rats. It focuses on a growing number of applications and progress in immunosensors for tumor markers. It covers the basic principles and biomedical and clinical applications of immunosensors, and indicates the future prospects in this field [39].

4. CONCLUSION

Hence we conclude that administration of diet rich in the natural products as chitosan is very important for treatment of different body organs, especially liver against liver disease and or even innate immunity.

5. REFERENCES

1. Lotze MT, Zeh HJ, Rubartelli A, Sparvero LJ, Amoscato AA, Washburn NR.2007. The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. Immunol Rev ; 220:60–8110.1111/j.1600-065X.2007.00579.x
2. Sushma, B.; Kanwaljit, C. and Praveen,R. 2010.Vitamin E Supplementation Modulates Endotoxin-induced Liver Damage in a Rat Model .Am. J. Biomed. Sci., 2(1), 51-62.
3. Cavalcanti, Anna Sylvia R. R. M.; Furtado, Gloria T.; Lisboa, Hugo M.; Fook, Marcus Vinicius L.2013.Morphological Characterization of Chitin ExtractionSource: Journal of Chitin and Chitosan Science, Volume 1, Number 2, September 2013, pp. 157-160(4)Publisher: American Scientific .
4. Malviya, R., P. Srivastava, M. Bansal P.K. Sharma, 2010. Preparation and Evaluation ofChitosan for prolonging anesthetic effect of this drug Disintegrating Properties of Cucurbita maximafor transdermal delivery. Lecithin was used as permeation Pulp Powder. And different molecular weights (MW) and concentrations of International J. Pharmaceutical Sci.,enhancer. Viscosity, bio-adhesion, drug release from 2(1): 395-399.
5. NRC(National Requirments of Laboratory animals)fourth revised edition.1995. subcommittee on laboratory AnimalNutrition , National Research Conuncil, National Academy Press,Washington , D.C.Of the lipid – lowering agents clofibrate and
6. Zhang, J., J. Liu, L. Li and W. Xia.2008. Dietary chitosan improves hypercholesterolemia in rats fed high fat diets Nutr Res., 28: 383-90.
7. Hussien, M.H.M. and Z. Abdeen.2008.Characteriz\tion and application of extracted natural polymer fiber(aminopolysaccharide) as weight reducing agent. The 3rd international conference of chemical industries. National Research Center, Cairo, Egypt.
8. Murray. 1984 Report on the symposium “drug effect in clinical chemistry methods .Eur J Clin Chem clin Biochem .34;385-386.
9. Bleta and Gella 1990. Report on the symposium “drug effect in clinical chemistry methods .Eur J Clin Chem clin Biochem .34;385-386.
10. Whicher, JT ;Warr0nc;chammber , R.E.1984.Immunochemical assay for Igs.Ann. Clin. Biochem ., 21: 78-91.
11. Stastical Analysis system (S.A.S).2002. Version 9.00 .S.A.S Institute Inc., cary , NC,USA
12. Adams LA, Angulo P.2005. Recent concepts in non-alcoholicfatty liver disease. Diabet Med 2005; 22: 1129-1133
13. Bayard M, Holt J, Boroughs E .2006."Nonalcoholic fatty liver disease" American Family Physician 73 (11): 1961–8.
14. Chavez-Tapia NC, Rosso N, Tiribelli C.2012.. Effect of intracellularlipid accumulation in a new model of non-alcoholicfatty liver disease. BMC Gastroenterol 12: 20 .
15. Jain,N.K.,Singhoi.,A.K.2011.protective effects of phullanthus acidus(l)skeels leaf extracts on acetaminophen and thoioacetamide induced hepatic injuries in wister rats . Asian pacific Journal of Tropical Medicine .4:470-474.
16. Yanpallewar, S.U.; Sen, S.; Tapas, S.; Kumar, M.; Raju, S.S. and Acharyal, S.B. 2002. Effect of Azadirachta indica on paracetamol-induced hepatic damage in albino rats. Phytomedicine; 9: 391-396.
17. A. L. Ahmad, N. H. Mat Yasin, C. J. C. Derek, and J. K. Lim,2011. “Optimization of microalgae coagulation process using chitosan,” Chemical Engineering Journal, vol. 173, no. 3, pp. 879–882.
18. Pradeep, K.; Mohan, C.R.; Gobianand, K.; Karthikeyan, S. 2007. Silymarin modul-ates the oxidant–antioxidant imbalance dur-ing diethylnitrosamine induced oxidative stress in rats. Eur. J. Pharmacol., 560 (2-3): 110-116.
19. Wang, Z., Huang, Y., Zou, J., Cao, K., Xu, Y., & Wu, J. M. 2002. Effects of red wine and wine polyphenol resveratrol on platelet aggregation in vivo and in vitro. International Journal of Molecular Medicine, 9(1), 77–79.
20. Radwan, R.R.; Shaban, E.A.; Kenawy, S.A. 2008. Hepatoprotective Efficiency of Combined Administration of Natural Antioxidants (Rutin and Vitamin E) and Cysteine in Hyperthermic Irradiated Rats. The Egyptian Journal of Hospital Medicine Vol., 32: 441– 454.

21. Sehrawat, A.; Khan, T.H.; Prasad, L. and Sultana, S. 2006. Butea monosperma and chemomodulation: Protective role against thioacetamide¬mediated hepatic alterations in Wistar rats. Phytomedicine; 13: 157-163.
22. Raghavendran, H.; Sathivel, A.; Devaki, T. 2004. Hepato-protective nature of seaweed alcoholic extract on acetaminophen induced hepatic oxidative stress. J. Health Sci., 50 (1): 42-46.
23. Fan, G, Jiang Jiang Tang, Monika Bhadauri, Satendr Kumar Niral, Fang Dai, Bo Zhou, Yang Li , Zhong Li Liu.2009. Resveratrol ameliorates carbon tetrachloride-induced acute liver injury in mice Environmental Toxicology and Pharmacology 28 ; 350–356
24. Schmatz, R.; Perreira, L.B.; Stefanello, N.; Mazzanti, C.; Spanevello, R.; Gutierres, J.; Bagatini, M.; Martins, C.C.; Abdalla, F.H. 2012. Effects of resveratrol on biomarkers of oxidative stress and on the activity of delta aminolevulinic acid dehydratase in liver and kidney of streptozotocin-induced diabetic rats Biochimie 94 ,374e383.
25. Guang-De Zhou, Mei-Rong Li, Jing Zhang, Deng Pan, Su-Xian Zhao, Jian-Fa Yang, Jun Yu and Jing-Min Zhao . 2008. Chitosan ameliorates the severity of steatohepatitis induced by high fat diet in rats. Vol. 43, No. 11 , Pages 1371-1377 .
26. Gallaher, C.M., J. Munion, Jr. R. Hesslink, J. Wise and D.D. Gallaher. 2000. Cholesterol reduction byglucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats. J Nutr., 130: 2753-9.
27. Li Z, Soloski MJ, Diehl AM.2005. Dietary factors alter hepatic innateimmune system in mice with nonalcoholic fatty liverdisease. Hepatology 42: 880-885
28. Jeong WI, Park O, Radaeva S, Gao B.2006. STAT1 inhibits liverfibrosis in mice by inhibiting stellate cell proliferationand stimulating NK cell cytotoxicity. Hepatology; 44:1441-1451
29. Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, PalùG, Martines D.2007. Increased intestinal permeability in obesemice: new evidence in the pathogenesis of nonalcoholic steatohepatitis.Am J Physiol Gastrointest Liver Physiol. 292;G518-G525 .
30. Korn T, Bettelli E, Oukka M, Kuchroo VK.2009. IL-17 and Th17Cells. Annu Rev Immunol; 27: 485-517 .
31. Kerkhoffs GM, Servien E, Dunn W, Dahm D, Bramer JA,Haverkamp D.2012. The influence of obesity on the complication rate and outcome of total knee arthroplasty: a meta-analysisand systematic literature review. J Bone Joint Surg Am;94: 1839-1844
32. Tarantino G, Savastano S, Colao A.2010. Hepatic steatosis, lowgradechronic inflammation and hormone/growth factor/adipokine imbalance. World J Gastroenterol; 16:4773-4783
33. Wouters K, van Gorp PJ, Bieghs V, Gijbels MJ, Duimel H, LutjohannD.2008. Dietary cholesterol, rather than liver steatosis, leads to hepaticinflammation in hyperlipidemic mouse models of nonalcoholic steatohepatitis. Hepatology;48:474-486.
34. Tangirala RK, Jerome WG, Jones NL, Small DM, Johnson WJ, GlickJM.2012. Formation of cholesterol monohydrate crystals in macrophage-derived foam cells. J Lipid Res.35:93-104.Heopatology, 56, No. 3, Bieghsetal. 903.
35. L Illum, , I Jabbal-Gill,M Hinchcliffe, A.N Fisher, S.S Davis. 2001. Chitosan as a novel nasal delivery system for vaccines. Advanced Drug Delivery Reviews. 51(1–3) 81–96 Nasal Vaccines.
36. Sevda Şenel, Susan J McClure., 2004. Potential applications of chitosan in veterinary medicine.Advanced Drug Delivery Reviews Volume 56, Issue 10, 23 June, Pages 1467–1480
37. Edel A McNeela, David O'Connor, Inderjit Jabbal-Gill, Lisbeth Illum, Stanley S Davis, Mariagrazia Pizza, Samuele Peppoloni, Rino Rappuoli, Kingston H.G Mills., 2004. A mucosal vaccine against diphtheria: formulation of cross reacting material (CRM197) of diphtheria toxin with chitosan enhances local and systemic antibody and Th2 responses following nasal delivery. Pharmaceutical Research 21(4): 671-674
38. Beong Ou Lim, Koji Yamada, Michiko Nonaka, Yuichiro Kuramoto,Pham Hung, and Michihiro Sugano. 2004. Dietary Fibers Modulate Indices of Intestinal Immune Function in Rats., Laboratory of Food Science, Kyushu University School of Agriculture, Higashi-ku, Fukuoka 812-81, Japan., Bioorganic & Medicinal Chemistry Letters. 11(13): 1699–1701.
39. Jie Wu Zhifeng Fu, Feng Yan, Huangxian Ju. 2007. Biomedical and clinical applications of immunoassays and immunosensors for tumor markersTrAC Trends in Analytical Chemistry . 26(7): 679–688.

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