



Hypolipidemic and anti-inflammatory effect of chitosan in experimental induced non-alcoholic fatty liver disease in rats

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ABSTRACT

The biochemical effect of Chitosan on hepatic lipid metabolism in experimental induced Nonalcoholic fatty liver disease (NAFLD) in rats was investigated. Thirty male albino rats were divided into three groups (10 rats each). Group I (control group): rats was fed on normal diet. Group II (NAFLD Group): rats fed on normal diet enriched with 1% cholesterol and 2% coconut oil. Group III (Chitosan treated group): rats fed on normal diet enriched with 1% cholesterol and 2% coconut oil and supplemented with Chitosan 5%. Blood samples were collected after 2, 4 and 6 weeks from the onset of chitosan treatment. All serum were directly used for determination of total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C), total lipids, (TL) Phospholipids, Non esterified fatty acids (NEFA), apo-lipoprotein -B (Apo-B), tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6). The obtained results showed a significant increase in serum TC, TAG, LDL-C, VLDL-C, TL, phospholipids, NEFA and Apo-B concentrations with a marked decrease in HDL-C level. Also, serum TNF- α , IL-6 was significantly increase in NAFLD group was observed. The results mentioned that treatment with Chitosan to NAFLD induced rats showed a significant change and ameliorate in all parameters nearly normal level that indicated the Hypolipidemic and anti-inflammatory effect of Chitosan in NAFLD induced rats.

Keywords: NAFLD, Chitosan, Lipid Profiles, pro-inflammatory cytokines

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1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is now recognized as the most common cause of cryptogenic cirrhosis. However, the diagnosis of cirrhosis in patients with NAFLD appears to be delayed compared with those with other chronic liver diseases and thus carries a higher mortality rate (Jeanne, et al., 2003). The accumulation of lipid droplets into the hepatocytes results in hepatic steatosis, which may develop as a consequence of multiple dysfunctions such as alterations in beta-oxidation, very low density lipoprotein secretion, and pathways involved in the synthesis of fatty acids. In addition an increased circulating pool of non-esterified

fatty acid may also to be a major determinant in the pathogenesis of fatty liver disease (Hamaguchi, et al., 2005). Among several mediators, cytokines and chemokines might play a pivotal active role in NAFLD and are considered as potential therapeutic targets, the evidence from both basic research and clinical studies on the potential role of cytokines and chemokines in the pathophysiology of NAFLD. Moreover, pro-inflammatory cytokines shift plays an important role in vascular regulatory mechanisms (Braunersreuther, et al., 2012).

It is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-

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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 (Cavalcanti, et al., 2013). Chitosan exhibits numerous health-related beneficial effects, including hypolipidemic, immunity regulation, anti-tumor, liver protection, anti-diabetic, antioxidant, anti-obesity, antibacterial and wound healing actions (Friedman and Juneja, 2010). Accordingly the aim of this study was to evaluate the hypolipidemic and anti-inflammatory effect of Chitosan in experimental induced NAFLD in rats.

2. MATERIALS AND METHODS

2.1. Animals and chemicals:

A total of thirty Male white albino rats, 6-8 weeks age and weighting (150–180g) were used in the experiment. Rats were housed in a separate metal cage with free access to water. Rats were kept under constant and nutritional environmental condition throughout the experimental period. Rats were left for 15 days before beginning of experiment for acclimatization.

Cholesterol and coconut oil were purchased from El-Goumhouria Co. for Trading Chemicals, Egypt.

NAFLD was induced by continuous supplementation of high fat diet (prepared by High Cholesterol (1% wt/wt) and (Coconut oil 2%wt/wt) to normal ration) according to NRC, 1995.

2.2. Chitosan:

Powder from crab shells poly-(1-4-β-D-glucopyranosamine), 2-Amino-2-deoxy β-D-glucopyranon (Cavalcanti et al., 2013). Chitosan was added to the basal diet at a dose 5% (1kg diet/50gm /Chitosan) according to Zhang et al., (2008).

2.3. Induction of NAFLD:

NAFLD induced by continuous supplementation of high fat diet (HFD)

containing high Cholesterol (1% wt/wt) and coconut oil (2%wt/wt) to normal ration according to (NRC,1995).after NAFLD induction treatment with Chitosan were given and continued for 12 weeks.

2.4. Experimental design:

Rats were divided into 3 main groups (10 per each) main groups classified as follow:

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 with Chitosan at a dose of 5% (1kg diet/50gm/Chitosan) for 12 weeks.

2.5. Sampling:

Samples were collected after overnight fasting from all animal groups (control and experimental groups) after 2, 4 and 6 weeks from the onset of treatment.

2.5.1. Blood samples:

Blood Samples were collected from Medial Canthes of eye and collected in dry, clean and screw capped tubes. Serum was separated by centrifugation at 2500 r.p.m for 15 minutes. The clean clear serum was removed by Pasteur pipette and kept in a deep freeze at -20C till used for determination of the following biochemical analysis :serum lipid profiles as TC, TAG, LDL-C, VLDL-C, HDL-C, total lipids, Phospholipids, NEFA, Apo-Band pro-inflammatory cytokines as serum TNF-α, IL-6 were determined according to the methods described by (Schettler and Nüssel,1975), (Friedewald,1972), (Bauer,1982), (Gordon,1977), (Kaplan and Natio,1984), (Connerty et al., 1961), (Schuster,1979), (Junger, 1998), (Beyaert and Fiers, 1998), (Chan and Perlstein, 1987) respectively.

2.5.2. Tissue Sample:

Liver specimens were preserved in 10% buffered neutral formalin and subjected for

Histopathological Examination according to the technique described by (Bancroft and Stevens, 1996)

2.6. Statistical analysis:

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

3. RESULTS

The results in tables (1,2and 3) revealed that, rats fed on high fat diet showed a significant increase in serum TC,TAG,LDL-C,VLDL-C, total lipids, Phospholipids, NEFA and Apo-B concentration while a significant decrease in serum HDL-C level was observed in NAFLD induced in rats after 2, 4 and 6 weeks when compared with the normal control group.

Administration of Chitosan to NAFLD induced rats exhibited a significant decrease in serum TC,TAG,LDL-C,VLDL-C, total lipids, phospholipids, NEFA And Apo-B concentrations with significant increase in serum HDL-C level in rats all the periods of experiment when compared with NAFLD non treated group .

The data in tables (1, 2 and 3) revealed that, rats fed hyperlipidemic diet revealed a significant increase in serum TNF- α andIL-6 concentrations in all the experimental periods compared with the normal control group.

Chitosan treatment exhibited a significant decrease in serum TNF- α and IL-6 concentrations when compared with control NAFLD non treated group.

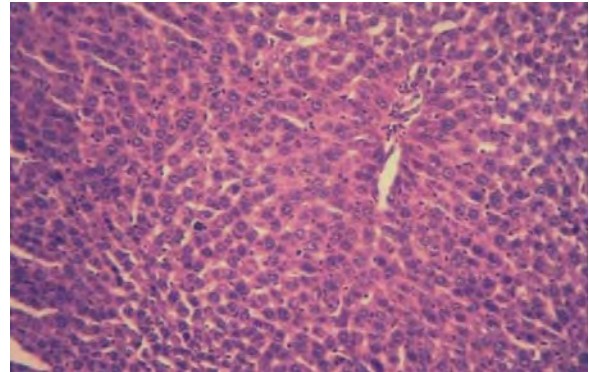


Figure (1): liver of normal control rats showing normal histological structure of the liver, normal hepatic lobules and hepatocytes, Group (1)

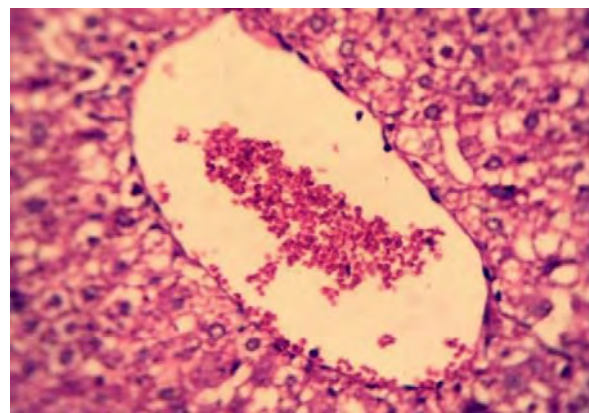


Figure (2): Liver of NAFLD rats, showing sever congestion of the central vein and portal blood vessels. The portal area showed mild hyperplasia of the epithelial cell lining of bile duct and mild fibrous tissue proliferation with Sever dilatation of central vein, Group II

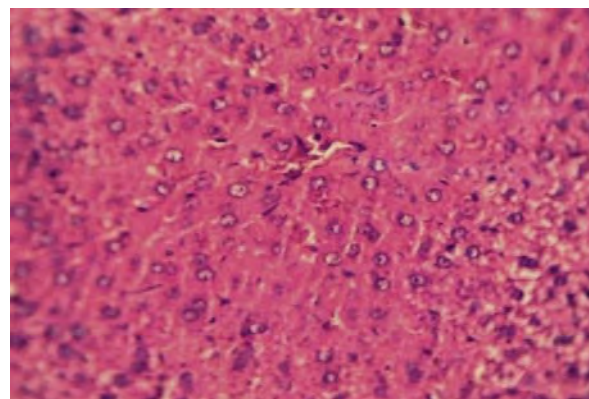


Figure (3): Liver of chitosan treated rats Group III, showing mild degree of degenerative changes in the hepatocytes. Mild congestion and mild vacuolar degeneration of hepatocytes.

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Table (1) Effect of two weeks treatment with Chitosan supplementation on serum lipid profiles and some pro-inflammatory cytokines in experimental induced non -alcoholic fatty liver disease in rats

Parameter/ group	T C mg/dl	TAG mg/dl	LDL- C .mg/dl	VLDL-C mg/dl	HDL- c mg/dl	Total lipid mg/dl	Phosholi pids mg/dl	NEFA ng/ml	ApoB ng/ml	TNF α Pg/ml	IL-6 Pg/m
Group I	89.18 ^e ±1.59	131.39 ^{de} ±14.55	19.28 ^C ±2.63	26.28 ^d ±2.91	40.40 ^C ±0.48	353.49 ^{cd} ±23.78	77.06 ^{bc} ±2.97	64.00 ^{cd} ±3.47	2.43 ^{cde} ±0.11	10.71 ^e ±1.06	43.87 ^{ab} ±1.51
Group II	149.05 ^{bc} ±22.35	204.62 ^{ab} ±13.29	59.25 ^{ab} ±16.09	35.72 ^a ±4.50	37.92 ^e ±2.33	490.73 ^{abc} ±35.40	106.84 ^a ±12.32	76.94 ^{cd} ±7.93	2.67 ^{bcd} ±0.21	51.27 ^a ±2.23	149.36 ^{ab} ±4.19
Group III	92.85 ^{de} ±2.99	194.05 ^{abc} ±8.29	21.76 ^e ±3.09	29.62 ^{cd} ±1.73	46.95 ^e ±1.84	377.29 ^{bcd} ±29.04	81.77 ^{bc} ±2.46	73.56 ^b ±2.34	2.81 ^{ab} ±0.08	12.10 ^e ±1.68	66.52 ^e ±2.58

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 ($P \leq 0.05$).

Table (2) Effect of four weeks treatment with Chitosan supplementation on serum lipid profiles and some pro-inflammatory cytokines in experimental induced non -alcoholic fatty liver disease in rats

Parameter/ group	T C mg/dl	TAG mg/dl	LDL- C .mg/dl	VLDL-C mg/dl	HDL- c mg/dl	Total lipid mg/dl	Phosholi pids mg/dl	NEFA ng/ml	ApoB ng/ml	TNF α Pg/ml	IL-6 Pg/m
Group I	83.04 ^e ±0.85	113.72 ^e ±8.69	16.49 ^C ±3.45	22.76 ^e ±1.74	41.65 ^C ±1.19	335.25 ^{cd} ±8.06	71.67 ^{bc} ±4.90	41.44 ^e ±2.46	2.16 ^e ±0.08	20.10 ^{cd} ±2.06	41.15 ^e ±2.85
Group II	192.29 ^a ±27.68	185.69 ^{abc} ±14.49	66.97 ^a ±16.84	34.47 ^{ab} ±3.49	35.69 ^e ±2.01	446.84 ^{ab} ±20.32	103.41 ^a ±9.24	61.87 ^{de} ±2.31	2.40 ^{cde} ±0.17	39.14 ^b ±1.42	165.91 ^a ±7.62
Group III	94.55 ^{de} ±3.66	96.75 ^e ±9.77	25.44 ^C ±4.26	19.55 ^e ±0.94	46.44 ^C ±2.76	309.30 ^e ±8.32	75.82 ^{bc} ±2.58	44.57 ^e ±2.15	1.41 ^{ef} ±0.10	21.64 ^{cd} ±1.18	66.26 ^e ±6.27

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 ($P \leq 0.05$).

Table (3) Effect of six weeks treatment with Chitosan supplementation on serum lipid profiles and some pro-inflammatory cytokines in experimental induced non -alcoholic fatty liver disease in rats

Parameter/ group	T C mg/dl	TAG mg/dl	LDL- C .mg/dl	VLDL-C mg/dl	HDL-c mg/dl	Total lipid mg/dl	Phosholi pids mg/dl	NEFA ng/ml	ApoB ng/ml	TNF α Pg/ml	IL-6 Pg/m
Group I	82.87 ^e ±6.75	159.02 ^{abc} ±5.46	24.18 ^c ±2.15	33.80 ^{ab} ±1.09	32.87 ^e ±0.70	353.19 ^{cd} ±4.88	66.01 ^c ±1.50	67.59 ^{bc} ±2.48	2.53 ^{cd} ±0.08	12.86 ^e ±2.14	63.86 ^a ±3.19
Group II	173.12 ^{ab} ±32.31	176.84 ^{abc} ±20.44	75.87 ^{ab} ±24.94	39.58 ^a ±4.20	25.69 ^{cd} ±1.82	364.44 ^{cd} ±23.58	90.15 ^{ab} ±23.58	63.98 ^{bc} ±9.41	2.65 ^{bcd} ±0.27	49.31 ^a ±4.65	127.87 ^{bc} ±11.96
Group III	101.16 ^{de} ±1.32	127.39 ^{de} ±5.39	26.03 ^C ±3.18	25.75 ^{de} ±1.13	48.50 ^C ±2.63	385.88 ^{bc} ±4.17	89.19 ^b ±2.98	49.47 ^{de} ±1.79	2.53 ^{cde} ±0.02	17.46 ^{de} ±2.67	20.09 ^{ef} ±0.80

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 ($P \leq 0.05$).

4. DISCUSSION

The obtained results demonstrated in tables (1, 2 and 3) revealed that, a significant increase in serum lipid profiles in experimental induced NAFLD when compared with normal control group. These results were nearly similar to Bradbury, et al., (2004) ; Tessari, et al., (2009) who reported

that, the increase of plasma triglyceride rich lipoproteins (TRLs) is associated with multiple alterations of other lipoproteins species that are potentially atherogenic has expanded the picture of diabetic dyslipidaemia. Moreover Taskinen, (2003) found that, a significant increased of lipid delivery to liver due to hepatic mitochondrial

metabolism is increased in subjects with nonalcoholic fatty liver disease subjects with increased IHTG had elevated adipose lipolysis which contributed to increased lipid delivery to liver. Hepatic TCA cycle flux was increased, indicating up regulated mitochondrial respiration (at least via complex II) and suggesting increased flux of acetyl-CoA from β -oxidation also due to Nonalcoholic fatty liver disease (NAFLD) is used to describe a condition of fat accumulation in the liver in the absence of excessive alcohol consumption and any other specific causes of hepatic steatosis. (Choi et al., 2014). However Quercioli et al., (2009) showed that, mRNA expression of enzymes involved in hepatic fatty acid oxidation (carnitine palmitoyl transferase1) and output (microsomal triglyceride transfer protein) the causal relationship between hepatic fat accumulation, insulin resistance, liver damage and the etiological role of hepatic fat accumulation in pathogenesis of extra- and intra-hepatic manifestations. On the other hand Janet et al., (2000) revealed that, a significant decreased in HDL-C was observed in experimental induced NAFLD in rats after 2,4 and 6 weeks compared with the normal control group. Such decreased may be due to improved solubility of the lipid and this increase due to catabolic effect of serum cholesterol. Additionally a significant decreased in HDL-C may be due to FFA, which are elevated in diabetes and insulin resistance, may also contribute to the increased production of reactive oxygen species (ROS) due to increased mitochondrial uncoupling and β -oxidation. (King and Loeken, 2004). In addition, increased circulating pool of non-esterified fatty acid may also to be a major determinant in the pathogenesis fatty liver disease because the Transcription factors such as sterol-regulatory-element-binding protein-1c and peroxisome proliferators activated receptor alpha, which promote either hepatic fatty acid synthesis or oxidation (Nguyen et al., 2007).

Treatment with Chitosan in NAFLD in rats exhibited a significant decrease in serum TC, TAG, LDL-C, VLDL-C, total lipids, phospholipids, NEFA and Apo-B with a significant increase HDL-C concentrations compared with NAFLD non treated groups. These results were nearly similar to Qin & Tian, (2010) who reported that, the significant decrease in lipid profiled may be due to Chitosan was interact with oil, which inhibited duodenal absorption and enhanced lipid excretion and the regulation of excessive lipid synthesis and uptake is thought to be an effective intervention for NAFLD. Thus, lipid-lowering agents are promising pharmacological therapies for hepatic steatosis (Zeng et al., 2008). On the other hand Guangfei et al., (2007); Guang-De Zhou et al., (2008) showed that Chitosan exhibited a significant decrease in serum levels of TC, LDL-C, TAG and increase fecal bile acids excretion but the levels of TG and HDL-C in plasma was unchanged. In addition, saturated fat and cholesterol fed could significantly induce the reduction of LDL receptor mRNA levels, while Chitosan could increase hepatic LDL receptor mRNA levels. Chitosan improve lipid metabolism by regulating TC and LDL-C by up regulating of hepatic LDL receptor mRNA expression, increasing the excretion of fecal bile acids and Chitosan a natural product derived from chitin, was thought to possess hypocholesterolemic properties. Chitosan effectively attenuated the steatohepatitis induced by a high fat diet. The therapeutic effect of chitosan on NASH may be activated through exerting an influence on adipokines. However, Maha et al., (2011); Paweł Wydro et al., (2007) revealed that the first mechanism the positive charges on Chitosan attract the negatively charged fatty acids and bile acids binding them to the indigestible chitosan fiber. This mechanism can explain why Chitosan reduces LDL cholesterol levels. Our bodies make bile acids in the liver using the cholesterol from LDL. When Chitosan binds

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bile acids it increases the rate of LDL loss thus improving the LDL to HDL ratio. If enough bile acids are bound, the fats are not solubilized, which prevents their digestion and absorption. The second mechanism describes a netting effect of Chitosan fiber. In this model the Chitosan wraps around fat droplets and prevents their being attacked and digested by lipid enzymes. Fats unprotected by Chitosan are digested and absorbed. The “netting” mechanism has been seen to operate in vivo. Substances that enhance the action of chitosan fibers can be likened to a tangled-up chain. Fibers must “unravel” in order for them to be of maximum benefit to us. “Unraveling” is especially critical for chitosan because each link has a hook on which to attach lipids. Chitosan can absorb an average of 4 to 5 times its weight in lipids. Fibers formulations can be prepared that unravel rapidly and swell quickly. These highly effective formulations are called super absorbants. When certain substances are added to chitosan, its remarkable fat-binding ability can be significantly enhanced the effects of chitosan on plasma and liver cholesterol levels, liver weight, and the key regulatory enzyme of cholesterologenesis 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase were observed in rats fed a sterol diet containing 1% cholesterol and 0.2% cholic acid, collectively indicate that the 7.5% chitosan formula maintained adequate cholesterol homeostasis in rats, despite a greatly increased intake of cholesterol. Furthermore Moghadasian et al, (2000); Arai et al (2000) stated that Chitosan is a safe and significantly reducing serum total cholesterol, especially in elderly women. However, the effect of chitosan on decreasing total cholesterol and LDL cholesterol is mild. On other hand, Fernandez, et al., (2001); Sabeena Farvin et al., (2007) recommended that , fiber sources into our diet may provide a useful adjunct to a low-saturated fat diet, and may have a further beneficial effect for individuals who have mild-to-moderate

hypercholesterolemia. The physicochemical properties of soluble fiber result in important modifications in volume, bulk and viscosity in the intestinal lumen, which will alter metabolic pathways of hepatic cholesterol and lipoprotein metabolism, resulting in lowering of plasma. Moreover Park et al.,(2007); Razdan et al., (2007) showed that, the use of chitosan to microencapsulate lipids and lipid-soluble components without compromising their bioavailability, although further human studies and Chitin-chitosan, when used as a food supplement, does lower plasma cholesterol and triglycerides and improves the HDL-cholesterol/total cholesterol ratio. Certain medical precautions, however, should be observed with long-term ingestion of high doses of chitosan to avoid potential adverse metabolic consequences may be due to the interrupt bile acid circulation, causing reduced lipid absorption and increased sterol excretion, which has also been observed in animal experiments. These results in accordance with Jiali Zhang et al., (2008); Fernandez et al., (2001) who showed that chitosan did not affect food intake but decreased body weight gain and significantly increased fecal fat and cholesterol excretion reduced the lipid level in plasma and liver, increased liver hepatic and lipoprotein lipase activities compared with HF and tended to relieve the degenerated fatty liver tissue. The physicochemical properties of soluble fiber result in important modifications in volume, bulk and viscosity in the intestinal lumen, which will alter metabolic pathways of hepatic cholesterol and lipoprotein metabolism, resulting in lowering of plasma. chitosan reduced the absorption of dietary fat and cholesterol in vivo and could effectively improve hypercholesterolemia in rats. Also Audrey et al., (2009) reported that, a significant decrease in triacylglycerol may be due to chitosan dietary fiber reduced lipid absorption. The lower triglyceridaemia observed upon chitosan treatment could also

be the result of the lower FIAF (fasting-induced adipose factor) expression observed in visceral adipose tissue. IL-6, resistin and leptin levels decreased in the serum after chitosan supplementation. Fungal chitosan counteracts some inflammatory disorders and metabolic alterations occurring in diet-induced obese mice since it decreases feed efficiency, fat mass, adipocytokine secretion and ectopic fat deposition in the liver and the muscle. In addition to Friedman and Juneja, (2010) suggested that, Chitosan has been shown *in vitro* to bind and precipitate micellar lipids including bile salts, cholesterol and triacylglycerol. Also, chitosan was reported to exhibit numerous health-related beneficial effects, including hypolipidemic, immunity regulation, anti-tumor, liver protection, anti-diabetic, antioxidant, anti-obesity, antibacterial and wound healing actions.

Moreover Guang-De Zhou et al., (2008) found that chitosan induced elevation in the levels of cholesterol, triglycerides, and free fatty acids in plasma and heart tissue of rats following myocardial infarction. It exerted an antilipidemic effect by reducing the level of low-density lipoprotein cholesterol with a parallel rise in the level of high-density lipoprotein cholesterol in plasma of experimental rats while chitosan elevated the serum levels of HDL. The therapeutic effect of chitosan on NASH may be activated through exerting an influence on adipokines. Regulation of excessive lipid synthesis and uptake is thought to be an effective intervention for NAFLD. (Sri Balasubashini, et al., 2003). However Razdan et al., (2007) stated that, chitin-chitosan, when used as a food supplement, does lower plasma cholesterol and triglycerides and improves the HDL-cholesterol/total cholesterol ratio (Davidson et al, 2002). The obtained results demonstrated in tables (1,2and3) showed a significant increase in serum TNF- α and IL-6 concentrations were observed in NAFLD induced in rats after 2, 4 and 6 weeks when

compared with the normal control group. These results were nearly similar to Hanrui Zhang et al., (2009) who suggests that, a significant increase in the inflammatory cytokine TNF- α plays a pivotal role in the disruption of macrovascular and microvascular circulation both *in vivo* and *in vitro*. advanced glycation end-products(AGEs) / receptor for AGEs (RAGE), lectin-like oxidized low-density lipoprotein receptor-1(LOX-1) and NF- κ B (nuclear factor κ B) signaling play key roles in TNF- α expression through an increase in circulating and/or local vascular TNF- α production. The increase in TNF- α expression induces the production of ROS (reactive oxygen species), resulting in endothelial dysfunction in many pathophysiological conditions. Lipid metabolism, dietary supplements and physical activity affect TNF- α expression. The interaction between TNF- α and stem cells is also important in terms of vascular repair or regeneration. Careful scrutiny of these factors may help elucidate the mechanisms that induce vascular dysfunction. On the other hand, Nappo et al.,(2002) found that a significant increased in TNF- α may be due to the mechanisms of TNF- α -induced endothelial dysfunction following high energy diets has been extensively studied at the molecular and cellular levels. Intra luminal butter administration significantly increased TNF- α expression in lamina propria macrophage and lymphocyte adherence to intestinal microvessels, accompanied by increases in the expression levels of ICAM-1, MAdCAM-1 (mucosal adhesion cell adhesion molecule-1) and VCAM-1. The high-fat meal, both triacylglycerol and TNF- α levels increased more in subjects with the metabolic syndrome than in normal subjects, whereas endothelial function decreased more in subjects with the metabolic syndrome the mRNA expression. Moreover the significant increase in serum interleukin-6 may be due to the leptin axis

has functional interactions with elements of metabolism, such as insulin, and inflammation, including mediators of innate immunity, such as interleukin-6. Leptin resistance and its interactions with metabolic and inflammatory factors, therefore, represent potential novel diagnostic and therapeutic targets in obesity-related cardiovascular disease (Seth et al., 2009). Moreover Lucero et al., (2009); Lotze et al., (2007) stated that, the activation of PRRs leads to cytokine production, contributing to liver injury and metabolic complications. In particular TNF α and IL-6, originally considered classical inflammatory cytokines, are now considered major links between steatosis, insulin resistance (IR), and related inflammatory disorders. Indeed, it has been demonstrated that TNF-reduced insulin signaling activation and its expression in liver is enhanced in patients affected by NAFLD who added that, in animal and human models, hepatic and serum IL-6 levels were higher in NAFLD. In addition to Corbould Anne, et al., (2014); Kerkhoffs, et al., (2012) revealed that 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 who found that, obese mice, after high fat and high cholesterol diets, express abnormal levels of macrophages and inflammation associated genes were observed in adipose tissue and in liver. Therefore Csiszar et al., (2007); Braunersreuther et al., (2012) showed that, a significant increase in serum TNF- α may be due to the dysregulation of TNF- α expression is associated with vascular aging, and anti-TNF- α treatment exerts anti-aging vasculoprotective effects. Aging is an independent factor in vascular dysfunction. In the presence of other risk factors, such as smoking and over-nutrition, the development of endothelial dysfunction might be

accelerated. risk factors converge on TNF- α to cause vascular oxidative stress, vascular remodelling, thrombosis, cell infiltration, apoptosis, vascular inflammation etc., and therefore lead to vascular damage and non-alcoholic steatohepatitis (the most inflamed condition in NAFLDs, which more frequently evolves towards chronic and serious liver diseases) is characterized by a marked activation of inflammatory cells and the up regulation of several soluble inflammatory mediators. Treatment with chitosan exhibited a significant decrease in serum TNF- α and IL-6 concentrations in experimentally induced NAFLD in rats compared with NAFLD non treated group. The significant decrease in serum IL-6 may be due to fungal chitosan counteracts some inflammatory disorders and metabolic alterations occurring in diet-induced obese mice since it decreases feed efficiency, fat mass, adipocytokine secretion and ectopic fat deposition in the liver and the muscle (Audrey et al., 2009). Moreover, Maha et al., (2011); Guang-De Zhou et al., (2008) revealed that Chitosan has an advantage in exerting a significant hepato-protective effect against non-alcoholic fatty liver. The hepato-protective effect of chitosan might be ascribable to its anti-inflammatory and/or antioxidant property. The present study demonstrated that, chitosan treatment provided an effective treatment against NAFLD in rats, since this compound was able to ameliorate serum lipid profiles and anti-inflammatory parameters.

Conclusion: we recommended that, administration of diet rich in Chitosan is very important for treatment of hyperlipidemia induced NAFLD and Inflammation of liver tissue.

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