



HYPOLIPIDEMIC ACTION OF CHITOSAN CORRELATES WITH ITS ANTIOXIDANT EFFECT AGAINST THIOACETAMIDE TOXICITY IN MALE ALBINO RATS

Shereen M. M. Yahya¹, Rokaya H. Shalaby¹, Fathia A. Mannaa², Noura R. Mohamed¹, Shereen H. B. Elwakeel^{1*} and Khaled G. Abdel-Wahhab²

¹Zoology Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

²Medical Physiology Department, National Research Centre, Dokki, Cairo 12622, Egypt.

***Corresponding Author: Shereen H. B. Elwakeel**

Zoology Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

Article Received on 06/08/2020

Article Revised on 26/08/2020

Article Accepted on 16/09/2020

ABSTRACT

Liver diseases represent a major health problem globally as its clearance function or elimination of toxins causes tissue damage. Chitosan is a safe natural constituent of bacteria and crustaceans exoskeleton. Thioacetamide (TAA) is one of the best well established liver toxic agent. The present study was conducted to evaluate the correlation between the hypolipidemic effect and the anti-oxidant activity of chitosan in TAA-induced toxicity in male Wistar rats. Fifty rats were divided into 5 groups and treated as follow; group1: served as control; group2 was injected with TAA (300 mg/Kg b.wt once); group 3 received 5% chitosan in diet for 14 days; group 4 fed 5% chitosan-diet for 14 days before TAA-injection; group 5 intoxicated with TAA prior to fed on 5% chitosan- diet for 14 days. TAA group showed deteriorated serum lipid profile pattern, decreased liver glutathione level and increased hepatic nitric oxide (NO) and lipid peroxidation (MDA) levels. However, feeding of rats on 5% chitosan-supplemented dies 14 days before or after TAA-intoxication showed ameliorated lipid profile levels close to normal levels, significantly increased hepatic glutathione level and significantly reduced liver NO and MDA levels. The correlation and linear regression analyses revealed a negative correlation among glutathione level and serum cholesterol, LDL-cholesterol and triglycerides levels while NO was positively correlated with these markers. These results highlighted that hypolipidemic action of chitosan could be mediated different mechanisms, mainly by its anti-oxidant property.

KEYWORDS: Thioacetamide; liver, chitosan; lipids; oxidative stress; rats.

INTRODUCTION

Liver disease is a main cause of morbidity and mortality. In 2017, World health organization reported that liver diseases represents one fifth of annual deaths in Egypt. Being responsible of xenobiotic metabolites, makes the liver subjected to continuous injuries from the environment. Liver continuous exposure to viruses, fat, alcohol, and biotransformed materials can cause liver injury which by turn results in liver inflammation and degeneration.^[1] Regardless of liver disease etiology, oxidative stress and inflammation are the key mechanisms of liver damage. However, oxidative stress and inflammation are not always a bad event; as they help phagocytes to trap and destroy pathogens and trigger signaling mechanism by redox modulations. Indeed, extended imbalance in the liver between the produced free radicals and their eliminations by antioxidant results in the damage of vital biomolecules within the cells.^[2]

Thioacetamide (TAA) was used extensively to induce acute and chronic liver toxicity in rats. It has a two-step

bioactivation process which is modulated by the microsomal (CYP) 2E1. The first metabolite is thioacetamide sulphoxide (TASO) which is further metabolized to an active metabolite thioacetamide-S-dioxide (TASO₂), a reactive metabolite that initiates cellular necrosis. TAA has the ability to activate nitric oxide synthase (iNOS).^[3] Additionally, it was reported that single intravenous injection of TAA alters liver biochemical and pathological pattern, in addition to the induction of pro-inflammatory cytokines changes. Besides TAA injection increased blood GOT, GPT, total bilirubin, direct bilirubin, NH₃ levels and decreased platelet, hemoglobin, and albumin levels in a dose dependent manner. Moreover, TAA injection increased white blood cells count, TNF- α and IL-6.^[4]

Many antioxidant therapies were proposed for protecting liver from oxidative stress.^[2] From the well established antioxidant agents is chitosan. Chitosan is a naturally occurring polysaccharide consisting of linking N-acetyl-2-amino-2-deoxy-D -glucopyranose (glucosamine) and 2-amino-2-deoxy-D-glucopyranose (N-acetyl-

glutamine) linked by different numbers of (1-4)-glycosidic bonds. Chitosan has gained much extensive studies as a biomedical substance. It showed a variety of biological activities such as antiallergic effects, anticoagulant effects, antitumor activities, immunostimulating effects, hemostatic agent, antimicrobial effects, hypocholesterolemic effects, anti-inflammatory activities, free radical scavenging activities, antibacterial effects, antiviral activity, antifungal activities and wound-healing.^[5] Chitosan was found to suppress hydroxyl radical, hydrogen peroxide, and the reactive oxygen species (ROS). Chitosan was found also to suppress membrane lipid peroxidation, or chelate the accumulated lipid peroxides. Moreover, chitosan have the ability to adsorb ion-exchange and chelate metal ions. Chitosan structure contains many hydroxyl groups (OH) and amino groups (NH) which mediates its antioxidant activity.^[6]

In spite of the well-established hypolipidemic and antioxidant actions of chitosan, yet no studies was conducted to test the correlations between the antioxidant activity and the hypolipidemic function. The current study was conducted to test the hypothesis that does the hypolipidemic action of chitosan is mediated by its antioxidant action. The hyperlipidemic and oxidative statuses were induced by the acute exposure to TAA which is a well-established hepatotoxin.

MATERIALS AND METHODS

Chemicals

Thioacetamide (TAA) was purchased from Sigma-Aldrich Co., (St Louis, Missouri, USA) as a pure crystal, it was dissolved in saline and freshly prepared prior to injection. Chitosan (CS) was collected from local Egyptian chemical shops. Cairo, Egypt. CS, an important polysaccharide of marine origin, is prepared from the shells of crustaceans and is being used as a new source of dietary fiber in drug development due to its intrinsic safety when taken orally. It was added to diet with 5% ratio.

Animals and experimental design

The experimental protocol of this study was approved by Ethics Committee of the National Research Centre (FWA 00014747) that follows the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals. Fifty adult male Wistar albino rats, weighing 120-150g, were obtained from the Animal House Colony, National Research Centre, Cairo, Egypt. The animals were housed in polypropylene cages in an environmentally controlled clean air room with a temperature of $25 \pm 1^\circ\text{C}$, an alternating 12h light/12h dark cycle, a relative humidity of $60 \pm 5\%$ and free access to tap water and a standard rodent chow. Rats were allowed to adapt to these conditions for two weeks before starting the experiment. After the acclimatization period, the animals were divided into five groups (10 rats each) as follow: Group (1) served as control and received saline solution as a vehicle for TAA, group (2) comprised of

healthy rats injected intraperitoneally with TAA dissolved in normal saline in a single dose (300 mg/kg b.wt) according to^[7] and fed standard basal diet for 14 days, group (3) comprised of healthy rats fed chitosan mixed basal diet at ratio of 5% (50g chitosan+950g basal diet) for 14 days according to the previous work of^[8] group (4) comprised of normal rats fed chitosan mixed basal diet at ratio of 5% for 14 days prior to intraperitoneal intoxication with a single dose (300 mg/kg b.w) of TAA dissolved in 0.9% normal saline, and group (5) comprised of normal rats fed chitosan mixed basal diet at ratio of 5% for 14 days after intraperitoneal intoxication with a single dose (300 mg/kg b.w) of TAA.

Body weight of each rat was recorded at begin and every week of the study period to monitor body weight change and to adjust the dose; at the end of experimental period, animals were fasted 12 hours and anaesthetized with diethyl ether; blood samples were collected from retro orbital venous plexus in centrifuge tubes, left to clot and cool centrifuged at 3000 rpm for 15 minutes; then the sera were separated, divided into aliquots and stored at -20°C for further biochemical analysis.

After blood collection, the animals were sacrificed by cervical dislocation, and the whole liver of each rat was rapidly excised and thoroughly washed with isotonic saline; then a portion of the liver of each animal was rolled in a piece of aluminum foil and frozen at -80°C for homogenization and determination of oxidative stress markers.

Preparation of liver homogenate

A specimen from the liver organ was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v) using ultrasound homogenizer; the homogenate was centrifuged at 5000 rpm for 20 minutes to remove the nuclear and mitochondrial fractions; the supernatant was divided into aliquots and stored at -80°C till the measurement of the markers of oxidative status as soon as possible.

Biochemical analysis

Serum triglycerides, total cholesterol, high density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) were determined spectrophotometrically using reagent kits obtained from Biodiagnostic Co, Egypt.

The level of malondialdehyde (MDA), the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems and used as an indirect index for lipid peroxidation, was determined chemically as described by^[9] through MDA reaction with thiobarbituric acid (TBA) forming a pink complex that can be measured photometrically; In this method, 0.5 ml liver homogenate supernatant was added to 4.5 ml working reagent (0.8 g TBA was dissolved in 100 ml perchloric acid 10%, and mixed with trichloro acetic acid 20% in a volume ratio 1 : 3, respectively);

then in a boiling and shaking water bath, the sample-reagent mixture was placed for 20 minutes, then carried to cool at room temperature and centrifuged for 5 minutes at 3000 rpm; finally, the absorbance of the clear pink supernatant was measured at 535 nm against reagent blank (0.5 ml distilled water + 4.5 ml working reagent); MDA level (nmol/g tissue) was calculated according to the following formula: $\text{MDA (nmol/g tissue)} = \left[\frac{A_{535} \times 10^9}{(1.56 \times 10^5) \times 10^3} \right] \times \text{AD} \times 10$. Where, $1.56 \times 10^5 \text{ M}^{-1}\text{L}^{-1}\text{cm}^{-1}$ is MDA extinction coefficient and AD is assay dilution (10).

Hepatic reduced glutathione (GSH) and nitric oxide (NO) levels were determined using conventional spectrophotometric technique and reagent kits obtained from Biodiagnostic Co, Egypt as guided by the manufacturer instructions.

Statistical Analysis

Data in the present study were expressed as mean $\pm$ SEM. Statistical significance of difference, at level of $p \leq 0.05$ was determined using analysis of variance (One way ANOVA) followed by post hoc comparisons using LSD test. Pearson's correlation was used to find correlations between the studied parameters.

RESULTS

As shown in Table (1), intoxication of rats with thioacetamide significantly increased the levels of serum total cholesterol and triglycerides, while rats fed chitosan-supplemented basal diet (5%) showed insignificant change in serum cholesterol and triglycerides levels when both groups were compared with the control group. Comparing with TAA-intoxicated rats group, the animals that fed chitosan-supplemented basal diet 14 days before or after intoxication with TAA group showed significant reduction in serum cholesterol and triglycerides levels.

Additionally, significant decrease in serum HDL level was detected in the TAA-intoxicated group, while animals' group fed chitosan-supplemented basal diet for 14 days showed insignificant change in serum HDL level when both groups were compared with the control group. Interestingly, feeding of rats on 5% chitosan-mixed basal diet 14 days prior to or after TAA-intoxication showed significant improvement in serum HDL level when both groups were compared with the TAA-intoxicated group.

Also, the data of this study revealed that feeding of rats on 5% chitosan-mixed diet didn't disturb serum LDL level, while TAA-intoxication resulted in a significant increase comparing both groups with the control one. Interestingly, feeding of rats on 5% chitosan-supplemented diet before or after injection with TAA led to significant decrease in serum LDL level, as compared with TAA-intoxicated group.

Table (1): Serum lipid profile of control, thioacetamide-intoxicated and chitosan-fed animals' groups.

	Control	TAA	Chitosan	Chitosan then TAA	TAA then chitosan
Cholesterol(mg/dl)	55.9 $\pm$ 1.3	85.2 $\pm$ 0.55 ^a	50.17 $\pm$ 0.9	62.6 $\pm$ 0.4 ^b	72.9 $\pm$ 0.45 ^b
HDL-Chol (mg/dl)	35.7 $\pm$ 1.5	20.3 $\pm$ 0.26 ^a	30.9 $\pm$ 0.7	31.5 $\pm$ 0.4 ^b	29.3 $\pm$ 0.44 ^b
LDL-Chol (mg/dl)	15.22 $\pm$ 2.08	50.12 $\pm$ 0.47 ^a	14.21 $\pm$ 1.01	21.97 $\pm$ 0.5 ^b	33.36 $\pm$ 0.48 ^b
Triglycerides (mg/dl)	34.9 $\pm$ 1.0	68.9 $\pm$ 0.58 ^a	35.3 $\pm$ 1.0	45.65 $\pm$ 0.8 ^b	51.2 $\pm$ 0.6 ^b

All data are presented as the mean $\pm$ SEM (n=10). Within the same raw, superscript letter "a" is a significant difference from control; while superscript letter "b" represents a significant difference from TAA-intoxicated group.

The results of this study illustrated that there was a significant depletion in hepatic GSH level in TAA-intoxicated group, while feeding of rats on 5% chitosan-supplemented diet showed insignificant change in hepatic GSH level as both groups were compared with the control group. Feeding of rats on chitosan-mixed diet 14 days before intoxication with TAA group resulted in a significant improvement in hepatic GSH level compared with TAA-injected group. Moreover, a significant improvement in GSH level was recorded in the group that fed chitosan-mixed diet after TAA-intoxication as both groups were compared with TAA-intoxicated group (Figure 1).

Furthermore intoxication of rats with TAA significantly increased the level of hepatic NO; while rats fed

chitosan-supplemented basal diet (5%) showed insignificant change in its level when both groups were compared with the control group. Comparing with TAA-intoxicated rats group, animals that fed chitosan-supplemented basal diet 14 days before intoxication with TAA group showed a significant reduction in serum NO level; meanwhile a significant decrease in serum NO level was recorded by animals' group that fed chitosan-supplemented basal diet 14 days after intoxication with TAA as shown in Figure (1).

The data illustrated in Figure (2) shows a significant rise in liver MDA level in the TAA-intoxicated group, while rats fed chitosan-supplemented basal diet (5%) showed insignificant change in serum MDA level in comparing both groups with the control one. In a

favorable manner, animals group that fed chitosan-supplemented basal diet 14 days before intoxication with TAA showed a significant reduction in liver MDA level, meanwhile a significant drop in its level was recorded in animals' group that fed chitosan-supplemented basal diet 14 days after intoxication with TAA.

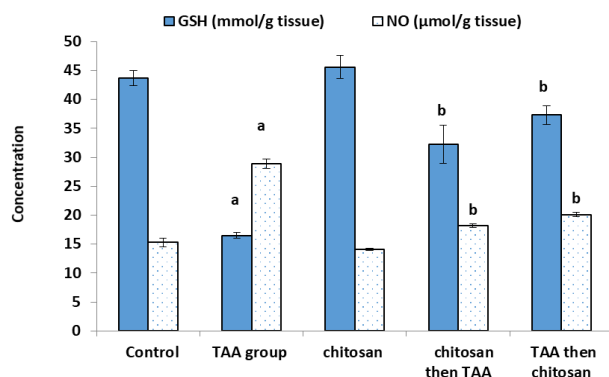


Figure (1). The effect of TAA-intoxication and 5% chitosan-supplemented basal diet on hepatic levels of GSH and NO (All the values are presented as the mean $\pm$ SEM). “a” is a significant difference from control; while superscript letter “b” represents a significant difference from TAA-intoxicated group.

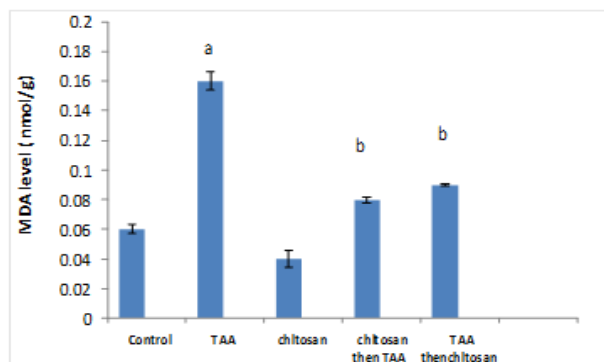


Figure (2). The effect of TAA-intoxication and 5% chitosan-supplemented basal diet on hepatic MDA level (All the values are presented as the mean $\pm$ SEM). “a” is a significant difference from control; while superscript letter “b” represents a significant difference from TAA-intoxicated group.

The data illustrated in Table (2) revealed a significant negative correlation among hepatic GSH level and each of serum total cholesterol ($r = -0.650$, $p = 0.0001$), LDL-c ($r = -0.700$, $p = 0.0001$) and triglycerides ($r = -0.711$, $p = 0.0001$) coupled with insignificant positive correlation with serum HDL-c in chitosan treated groups. Similarly, a significant positive correlation was noticed among liver nitric oxide and serum total cholesterol ($r = 0.793$, $P = 0.0003$), LDL-c ($r = 0.753$, $p = 0.001$) and triglycerides ($r = 0.789$, $p = 0.0001$), matched with insignificant negative relation with HDL-c ($r = -0.394$, $p = 0.146$). Hepatic level of MDA recorded insignificant positive relation with serum total cholesterol, LDL-c, and triglycerides, associated with insignificant negative relation with HDL-c.

Table (2). Correlation among oxidative stress markers (GSH, NO and MDA) and lipid profile indexes (total cholesterol, HDL-c, LDL-c and triglycerides).

	Total Cholesterol	HDL-c	LDL-c	Triglycerides
GSH	R = - 0.650	R= 0.350	R = - 0.700	R= - 0.711
	P= 0.0001	P = 0.058	P = 0.0001	P = 0.0001
	Sig	NS	Sig	sig
NO	R =0.793	R = - 0.394	R=0.753	R=0.789
	p = 0.0003	p = 0.146	P =0.001	P =0.0001
	Sig	NS	sig	Sig
MDA	R = 0.326	R= - 0.347	R= 0.338	R=- 0.323
	p = 0.078	p = 0.060	p = 0.068	p= 0.082
	NS	NS	NS	NS

DISCUSSION

Depending on its promising biological activities, chitosan was used extensively in many studies to propose a therapeutic option for different liver diseases. In the current study, an acute liver toxicity was induced using thioacetamide which is a well-established hepatotoxin. The present study aimed to evaluate the protective and curative efficiency of chitosan against lipid distortion as a complication of thioacetamide-induced hepatotoxicity. Besides the anti-lipidemic potential, the oxidative stress statuses as well as their correlation were investigated. TAA-intoxication deteriorated lipid profile in rats where

total cholesterol, triglycerides and LDL-c were significantly increased while HDL-c was significantly decreased. These finding is in accordance with^[10] and^[11]. The deteriorated lipid profile may be a result of liver toxicity which resulted in deteriorated lipid metabolic function. In case of deteriorated liver function, liver is disabling to metabolize lipids leading to hepatocyte death. However, feeding of rats on chitosan-mixed diet either as preventive (before TAA-intoxication) or curative (after TAA-intoxication) resulted in a marked amelioration in serum lipid pattern where cholesterol, triglycerides, and LDL-c levels were decreased with

concomitant increase in HDL-c level. These findings are consistent with,^[12],^[13]^[8] and.^[14] The later found that chitosan was able to reduce the absorption of dietary fat and cholesterol *in vivo*, this in turn ameliorated hyperlipidemia and fatty liver in the rats. Other vary mechanisms of chitosan mediated cholesterol lowering effect were postulated; one of them suggests that chitosan may affect cholesterol metabolism, independently of absorption and synthesis, another mechanism implies that chitosan exerts its action based on general characteristics of fibers which cause delayed gastric emptying, reduction in appetite, increased satiety, and modulation of secretion; by its capability of forming a high viscous gel, chitosan cause delaying of gastric emptying leading to increased satiety. Another mechanism is based on the antibacterial effects of chitosan which affect colonic fermentation result in increased short chain fatty acid production, leading to reduced cholesterol synthesis via propionic acid. Moreover, one mechanism depends on the established chitosan antioxidant activity.^[15]

Moreover, chitosan was found to increase hepatic LDL - c receptor through the up regulation of LDL - c mRNA receptor expression in rat liver leading to improved liver function and decreased cholesterol levels. Depending on its strong positive charge, chitosan binds to fatty acids and bile acids, which are negatively charged. Bile acids are produced from LDL-c in the liver. So, chitosan binding to bile acids enhances LDL -c loss and consequently LDL- c to HDL- c ratio is improved. The more the binding of chitosan and bile acids, the lesser the fat are solubilized which prevents its digestion and absorption. Another mechanism suggests that chitosan forms a net using its fibers and wraps around fat droplets preventing its digestion by lipid enzymes.^[12]

On the other hand, results of the current study revealed that TAA-injection resulted in a significant reduction in hepatic GSH level concomitant with significant elevations in liver Nitric oxide (NO) and MDA levels. GSH depletion was reported to be initiation factor that facilitate lipid peroxidation and finally increase MDA levels. NO forms free radicals, peroxynitrites, which is capable of peroxidizing the protein, LDL- c, and may affect other biological molecules. Peroxynitrites enhances the activation of poly (ADP-ribose) polymerase (PARP-1) resulting in up regulating the expression of some inflammatory genes. The increased inflammatory response increases the risk of cell death by the reduction of NAD⁺ and ATP cytosolic stores.^[16] Promisingly, fed on chitosan as protective or curative regimens significantly elevated hepatic GSH levels and reduced liver NO and MDA levels. Lipid peroxidation, inflammation and necrosis of hepatocytes could be suppressed by GSH.^[17] GSH can modulate its antioxidant functions in many ways; it may act directly as a free radical scavenger by reacting with free oxygen, superoxide and hydroxyl radical; besides, it could stabilize membrane structure through acyl peroxides

eradication.^[18]^[19]

Reported that chitosan has a strong positive charge and consequently it could bind to cell membranes and initiate important biological interactions with cell membrane. The currently established antioxidant properties of chitosan is in accordance with the findings of^[20-22] and.^[8] Antioxidants can inhibit the damaging effects of reactive oxygen species on protein, lipids and DNA in addition to preventing the formation of free radicals.^[23]

Moreover, it was reported that chitosan ingestion significantly inhibited liver thiobarbituric acid reactive substances and increased catalase and superoxide dismutase as an endogenous antioxidant enzymes.^[21] Besides, it was reported that hydroxyl radicals lipid peroxidation of phosphatidylcholine and linoleate liposomes was suppressed by the scavenging effect of chitosan *in vitro*.^[24] Additionally, chitosan is capable to scavenge hydroxyl and oxygen radical.^[25]

Negative correlation among hepatic glutathione level and serum cholesterol, LDL-c and Triglycerides levels was detected in this study; this findings are in accordance with^[15,26] who postulated that chitosan-lipids lowering effect depends on the established chitosan antioxidant activity. This may partially be consistent with the finding of^[27] who reported that HDL-c concentration negatively correlates with the concentrations of MDA and LDL-c which is the major source of oxidized LDL-c and is more atherogenic than LDL-c. Similarly,^[28] found that high level of MDA is associated with low level of HDL-c and high level of LDL-c. As mentioned previously, low GSH level leads to oxidative stress and consequently increased lipid peroxidation and MDA level. Moreover, NO was negatively correlated with total cholesterol, and correlated positively with LDL-c and triglycerides levels. This could be explained as a negative feedback mechanism of NO to dilate the blood vessels. This could suggest that chitosan anti-oxidant activity is significantly associated with its hypolipidemic effect in TAA-treated rats.

CONCLUSION

Our study concluded that chitosan possesses a markable lipid lowering potential as it succeeded in lowering non-useful serum lipid indexes and restoring the antioxidant battery in the hepatic tissues of the thioacetamide-induced acute hepatotoxicity. The liver antioxidant markers and serum lipid profile indexes have different significant correlations in chitosan-fed rats groups. All together suggest that the hypolipidemic effect of chitosan is mediated mainly by its antioxidant activity.

CONFLICT OF INTEREST: None of the authors have any competing interests in the manuscript.

FUNDING SOURCES: Our study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

- Grattagliano I, Bonfrate L, Catia VD, Wang HH, Wang DQH, Portincasa P. Biochemical mechanisms in drug-induced liver injury. *World Journal of Gastroenterology*, 2009; 15: 4865–4876.
- Li SS, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y. The Role of Oxidative Stress and Antioxidants in Liver Diseases. *Int J Mol Sci*, 2015; 16(11): 26087–26124.
- El-Baz FK, Salama A, Salama RAA. Therapeutic Effect of *Dunaliella salina* Microalgae on Thioacetamide- (TAA-) Induced Hepatic Liver Fibrosis in Rats: Role of TGF- β and MMP9. *Hindawi BioMed Research International*, 2019; 7028314: 9. pages <https://doi.org/10.1155/2019/7028314>
- Chen TM, Subeq YM, Lee RP, Chiou TW, Hsu BG. Single dose intravenous thioacetamide administration as a model of acute liver damage in rats. *Int. J. Exp. Path*, 2008; 89: 223–231.
- El-Denshary ES, Aljawish A, El-Nekeety AA, Hassan NS, Saleh RH, Rihn BH, Abdel-Wahhab MA. Possible Synergistic Effect and Antioxidant Properties of Chitosan Nanoparticles and Quercetin against Carbon Tetrachloride-Induce Hepatotoxicity in Rats. *Soft Nanoscience Letters*, 2015; 5: 36–51.
- Avelelas F, Horta A, Pinto LFV, Marques SC, Nunes PM, Pedrosa R, Leandro SM. Antifungal and Antioxidant Properties of Chitosan Polymers Obtained from Nontraditional *Polybius henslowii*. *Mar. Drugs*, 2019; 17: 239. doi: 10.3390/md17040239
- Mustafa HN, El Awdan SA, Hegazy GA. Protective role of antioxidants on thioacetamide-induced acute hepatic encephalopathy: Biochemical and Ultrastructural study. *Tissue Cell*, 2013; 45(5): 350–362.
- Abo Zaid OR, Abdel-hamid OM, Atwa SAE. Hypolipidemic and anti-inflammatory effect of chitosan in experimental induced non -alcoholic fatty liver disease in rats. *Benha Veterinary Medical Journal*, 2015; 28(1): 155–165.
- Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron- induced lipid peroxidation of rat liver mi- crosomes. *Steroids*, 1994; 59(6): 383–388.
- El-Kholy WM, El-Habibi EM, El-Megrabi R. AMELIORATING EFFECTS OF MARJORAM LEAVES EXTRACT AGAINST HEPATIC DISORDER IN MALE RATS. *Int. J. Adv. Res*, 2018; 6(5): 613–622.
- Kabiri N, Ahangar-Darabi M, Setorki M, Rafieian-kopaei M. The effect of silymarin on liver injury induced by thioacetamide in rats. *J Herb Med Pharmacol*, 2013; 2(2): 29–33.
- Guangfei X, Xiaodong H, Lianglin Q, Jinbiao W, Yinqing H. Mechanism study of chitosan on lipid metabolism in hyperlipidemic rats. *Asia Pac. J. Clin.Nutr*, 2007; 16(1): 313–317.
- Zhou GD, Li MR, Zhang J, Pan D, Zhao SX, Yang JF, Yu J, Zhao JM. Chitosan ameliorates these verities of steatohepatitis induced by high fat diet in rats, 2008; 43(11): 1371–1377.
- Wang Z, Zhang F, Yan Y, Zhang Z, Wang L, Qin C. Lipid-lowering activities of chitosan and its quaternary ammonium salt for the hyperlipidemia rats induced by high-fat diets. *Int J Biol Macromol*, 2019; 132: 922–928. doi: 10.1016/j.ijbiomac.2019.03.214. Epub 2019 Mar 28.
- Dieter Lütjohann D, Marinova M, Wolter K, Willinek W, Bitterlich N, Coenen M, Coch C, Stellaard F. Influence of Chitosan Treatment on Surrogate Serum Markers of Cholesterol Metabolism in Obese Subjects. *Nutrients*, 2018; 10(1): 72.
- Carlos KB, Ferrari, Eduardo Luzía França, Adenilda Honorio-França. Nitric oxide, health and disease. *Journal of applied biomedicine*, 2009; 20(4): 163–173.
- Jia R, Cao L, Xu P, Jeney G, Yin G. In vitro and in vivo hepatoprotective and antioxidant effects of *Astragalus polysaccharides* against carbon tetrachloride-induced hepatocyte damage in common carp (*Cyprinus carpio*). *Fish Physiol Biochem*, 2012; 38: 871–881.
- Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal*, 2016; 15: 71. DOI 10.1186/s12937-016-0186-5.
- Hasegawa M, Yagi K, Iwakawa S, Hirai M. Chitosan induces apoptosis via caspase-3 activation in bladder tumor cells. *JpnJ Cancer Res*, 2001; 92: 45966.
- Chiang MT, Yao HT, Chen HC. Effect of dietary chitosans with different viscosity on plasma lipids and lipid peroxidation in rats fed on a diet enriched with cholesterol. *Biosci. Biotechnol. Biochem*, 2000; 5: 965–971.
- Mohamed NE. Effect of Chitosan on Oxidative Stress and Metabolic Disorders Induced In Rats Exposed to Radiation. *Journal of American Science*, 2011; 7(6).
- Ozcelik E, Uslu S, Erkasap N, Karimi H. Protective effect of chitosan treatment against acetaminophen-induced hepatotoxicity, 2014; 30(6): 286–90. doi: 10.1016/j.kjms.2014.02.003. Epub 2014 Apr 3.
- Boojar MM, Shockravi A. On the cytotoxicity and status of oxidative stress of two novel synthesized tri-azamacrocyclicdiamides as studied in the V79 cell lines. *Bioorg.Med.Chem*, 2007; 15(10): 3437.
- Xie W, Xu P, Liu Q. Antioxidant activity of water soluble chitosan derivatives *Bioorg.Med. Chem.Lett.*, 2001; 11: 1699–1701.
- Harish P, Dharmesh KV, Jagannatha Rao SM, Tharanathan KS, Rudrapatnam N. Free radical induced chitosan depolymerized product protect calf thymus DNA from oxidative damage. *Carbohydrate Research*, 2007; 342(2): 190–195.

26. Avelelas F, Horta A, Luís FV, Pinto LFV, Marques SC, Nunes PM 1, Pedrosa R, Leandro SM. Antifungal and Antioxidant Properties of Chitosan Polymers Obtained from Nontraditional *Polybius henslowii* Sources. *Mar. Drugs*, 2019; 17: 239.
27. Kondo A, Muranaka Y, Ohta I, Notsu K, Manabe M, Kotani K, Saito K, Maekawa M, Kanno I. Relationship between triglyceride concentrations and LDL size evaluated by malondialdehyde-modified LDL. *Clin Chem*, 2001; 47(5): 893-900.
28. Moriyama K, Takahashi E. Evaluation of Malondialdehyde Low-Density Lipoprotein Stratified by Low-Density Lipoprotein Cholesterol. *Clin Lab*, 2017; 63(7): 1179-1186. doi: 10.7754/Clin.Lab.2017.170113.