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Antioxidant and Hepatoprotective Effects of Chitosan on High Fructose Induced Liver Damage in Albino Rats

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Abstract

Chitosan is a biodegradable natural polymer with many advantages such as nontoxicity, biocompatibility, and biodegradability. The current study aimed to evaluate the role of chitosan in reducing or regulating liver disorders caused by high fructose.concentration. The study was performed on thirty-two Wistar male rats, weighing between 195-200gms. These rats divided into four groups: control group which was kept on distill water, group 2 (G2) dosed with 40% fructose, group 3 (G3) dosed with 250 mg/kg of chitosan and group 4 (G4) dosed with 40% fructose and 250 mg/kg chitosan. After four weeks of study the blood samples were taken by heart puncturing and then all animals were sacrificed and specimens from liver were taken and homogenized for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase activity (ALP), and superoxide dismutase (SOD) determination. Liver tissues blocks were prepared by paraffin method stained using hematoxylin and eosin (H&E) stain. Highest concentration of ALT, AST, ALP, and SOD was detected in group G2 while serum albumin level decreased in rats in that group. We also found clear histological disorders in the liver of rats of this group. A reduction in the liver damage in G4, as the role of chitosan (G3) in improving liver function was proven by converging the level of the studied indicators from its counterpart in the control group. It can be concluded that chitosan is a successful protective agent for liver function: as it reduced hepatic impairment resulting from the use of fructose.

Keyword: Chitosan; Fructose; Liver; Antioxidant; Histopathology

تأثيرات الكيتوسان المضاد للأكسدة و الواقية لتلف الكبد المحفز بالتركيز العالي للفركتوز لدى ذكور الجرذان

مسار جبار الكردي و مريم عطية خزعل

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الخلاصة

الكيتوسان عبارة عن بوليمر طبيعي قابل للتحلل الحيوي وله العديد من المزايا مثل عدم السمية , التوافق الحيوي وقابلية التحلل الحيوي. هدفت هذه الدراسة إلى تقييم دور الكيتوسان في تقليل أو تنظيم اضطرابات الكبد الناتجة عن ارتفاع تركيز الفركتوز. شملت هذه الدراسة على 32 ذكور جرذ ألبينو تم تقسيمهم إلى أربع مجموعات تم إعطاء كل مجموعة جرعات محددة تمثلت بجرعة واحدة يوميا حتى 4 أسابيع على النحو التالي

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؛ المجموعة الضابطة (G1) التي تم تجريعها بالماء المقطر ، المجموعة 2 (G2) تم تجريعها وبالفركتوز 40٪ ، المجموعة 3 (G3) تم تجريعها ب 250 مجم / كجم من الكيتوسان والمجموعة 4 (G4) جرعت ب 40٪ من الفركتوز و 250 مجم / كجم من الكيتوسان. باستخدام عدد الفحص التجارية تم قياس نشاط إنزيم ناقلة الألانين (ALT)، انزيم ناقلة الأسبارتات (AST) ، سوبر اوكساید ديسموتاز (SOD) وانزيم الفوسفاتاز القلوي (ALP) في المصل. تم اكتشاف اعلى تركيز ل ALT و AST و ALP و SOD في المجموعة G2 بينما انخفض مستوى ألبومين المصل في الفئران في تلك المجموعة ، كما وجدنا اضطرابات نسيجية واضحة في كبد جرذان هذه المجموعة. في حين ، لاحظنا انخفاضًا في تلف الكبد في المجموعة G4 ، فقد تم إثبات دور الكيتوسان في تحسين وظائف الكبد من خلال تقارب مستوى المؤشرات المدروسة في المجموعة G3 من نظيرتها في المجموعة الضابطة. في الختام: الكيتوسان عامل وقائي ناجح لوظيفة الكبد من خلال تقليل القصور الكبدي الناتج عن استخدام الفركتوز.

1. Introduction

The regulation of blood sugar levels and the elimination of toxins are two of the most crucial functions performed by the liver, a major organ in the body [1]. These functions may be lost if the liver is damaged or infected with a virus, which can cause significant harm to the body [2]. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase activity (ALP), superoxide dismutase (SOD), and catalase (CAT) are just some of the biochemical compounds that have been shown to be suppressed by antioxidants to prevent hepatotoxicity [3],[4]. Numerous recent scientific studies have investigated the antioxidants effects on liver disease treatment and their support for normal liver function. In recent years, antioxidants have received a lot of attention as useful alternative medicines for treating or preventing disorders related to lifestyle. However, very little is known about their method of operation [5],[6].

Chitosan is a marine-derived polysaccharide made from crustacean shells. Due to its antitumor, anti-ulcer, immunostimulant, and antibacterial properties, it has received a lot of attention as a biomedical material [7],[8]. Chitosan has unique properties that make it useful for chelating, forming membranes, and multicellular applications as it is an oxygen-rich linear polysaccharide with active amino and hydroxyl groups. As a result, chitosan possesses a number of intriguing biological properties, including biocompatibility, biodegradability, non-toxicity, non-permeability, and sorption [9],[10].

Fructose is a naturally occurring monosaccharide that was first isolated from sugar cane in 1847. It is found in many vegetables, fruits, and grains. It is the predominant sugar in apples, grapes, oranges and melons, and constitutes up to half of the total sugars in honey [11,12]. Fructose is a component of othercarbohydrates, such as the disaccharide sucrose,and is thus a constituent in manyoligosaccharides and polysaccharides thatcontain sucrose [13]. Additionally, fructose is thechief component in the fructan polysaccharides,levan and inulin [3]-[5],[14]. Such fructanpolysaccharides are being increasingly utilized inthe health food and pharmaceutical industries [14]. On other hand, high consumption of fructose leads to many health problems. At the physiological level, the liver can completely metabolize fructose. However, excessive fructose consumption promotes lipogenesis, dyslipidemia, visceral adiposity, and insulin resistance, all of which contribute to steatosis. Additionally, it has been discovered that both non-alcoholic steatohepatitis and non-alcoholic fatty liver disease are more likely to occur in people who consume high fructose corn syrup and other sugars like sucrose [15],[16]. After evaluating those problems, the current study attempted to reduce the pathogenic effect of fructose on the liver by using chitosan and by conducting an experimental study on male rats model, in which liver disorders were stimulated using high

concentrations of fructose and testing the effectiveness of chitosan in correcting the disturbance in liver function.

2. Materials and Methods

2.1. Experimental Animals

Thirty-two adult Wistar male rats weighing 195-200 grams and about 5-7 weeks old were used in this experiment. The rats were purchased from The Animal Research Center, College of Veterinary Medicine, University of Baghdad. After the study protocol was approved by ethics committee (ethics number: 118 in 16/3/2022), rats were housed for 10 days according to standard guidelines for care and use of laboratory animals including 22-25 ℃ with 12 hours' light/dark cycle and a relative humidity of 50-70%. in clean polypropylene cages. They were fed a steady supply of appropriate laboratory formula.

2.2. Chemical Compounds Preparation

According to Mamikutty *et al.* [17], the fructose solution was made by dissolving 20 grams of pure fructose crystalline purchased from Pharmacy for Medical Products and Materials in Baghdad, Iraq, in 100 milliliters of deionized water (20 percent w/v). Sigma Chemical Co. (St. Louis, MO, USA) procured chitosan and prepared it in accordance with Ozcelik*et al.* [18].

2.3. Design of the Experiment and Collection of Samples

The duration of the current study was four weeks. Rats were randomly divided into four groups with eight rats in each group:

G1: Rats in control group were kept on distill water during experiment period.
G2: Rats in this group were proved orally with 40% (2.5 mm/g) fructose once daily.
G3: Rats in this group were provided orally with 250 mg/kg/oral chitosan once daily.
G4: Rats in this group were received 40% fructose (2.5 mm/g) plus 250 mg/kg chitosan once a day.

Samples Collection

Animals, after 12 hr fasting, were deeply anaesthetized with chloroform., blood samples were collected from a cardiac vein puncture in sterile, heparin-free tubes, and the serum was separated by centrifugation at 4000 rpm for 10 minutes. Automatic pipettes were used to collect the serum, which was stored in sterile tubes at -20°C until used.

2.4. Biochemical Analysis

Serum level of ALT, AST, ALP and Albumin was analyzed by enzymatic kits manufactured by Agappe, India. After euthanizing rats, specimens from liver were taken, washed in saline in an ice bath and homogenized for determination of superoxide dismutase (SOD). The commercially available SOD assay kit protocol was followed for measuring the SOD activity.(SOD Elabscience® ELİSA Kit, USA). Moreover, the catalase (CAT) activity was assayed using a commercially available assay kit (CAT E1abscience®/ELISA Kit, USA).

2.5. Histopathological Preparation

Following standard histological procedures, liver tissues reserved for histopathological examination were fixed with 10% neutral-buffered formalin and embedded in paraffin. The paraffin was cut into sections of 4 µm thickness using microtome, and stained with hematoxylin and eosin (H&E) solution. Tissues were subsequently mounted and cover slipped using mounting medium and then examined microscopically (Olympus, Tokyo, Japan). Three sections in each group were used for this purpose [25].

2.6. Statistics

Statistical Package for the Social Sciences (SPSS) version 19 and Excel 2010 were used to analyze the data statistically. ANOVA test was performed to find out the significant differences in the serological and biochemical indicators between the four groups., while t-test was used to find out the significant differences in these indicators between the two groups. The probability value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Biochemical results

To evaluate fructose and chitosan effects on the body in general, animals were weighted at the beginning and end of the experiment (Table 1). No effect of these carbohydrates on body weight at the beginning of the experiment ($p > 0.05$). however, at the end of the experiment a decrease in the animals weight was recorded in G 2 which was dosed with fructose alone when compared with rats in the other groups ($p < 0.05$), where the lowest gain in weight (17.87 g) was noticed in that group. On the other hand, the liver was also weighted at the end of the experiment, and a decrease in its weight was observed in G2, which was accompanied by significant differences when compared with liver weight in the rest of the groups ($p<0.05$) (Table 2).

To know the role of chitosan in protecting the liver and regulating its functions, we created damage to liver function by fructose sugar. To evaluate the effectiveness of chitosan in protecting the liver, the following indicators were studied ; ALT, AST, ALP, S0D and CAT. In Table 3 and Figures 1&2 the highest concentration of ALT, AST,ALP, S0D and CAT can be seen in rats that were fed fructose only in group G2 (100.5U/I, 172.2 U/I, 119.6 U/I, 32.99 U and 324.8Urespectively), where fructose increased the level of these indicators as a result of an increase in oxidative stress level and inflammation. Whereas, chitosan, when given with fructose (group G4), slightly reduced the rise in these indicators (71.27 U/I, 134.1 U/I, 72.16U/I, 17.92U and 286.7 U respectively)while the average concentration of ALT (66.95 U/I), AST (127.4 U/I), ALP (72.01 U/I), and SOD(13.54U) in rats treated with chitosan only (group G3) was close to or slightly higher than the control group G1 (65.82 U/I, 125.53 U/I, 71.27 U/I and 11.99U respectively). It was also detected that fructose decreased the serum albumin level in rats in the G2 group (1.41 mg/dL) and G4 group (2.07 mg/dL), while it was elevated in the rats that were dosed with chitosan in the G3 group (2.44 mg/dl) and the control group (2.43 mg/dl). On the other hand, a random variation in the concentration of CAT was observed between the four groups ($P<0.05$), as its concentration increased in groups G3 (262.2 U) and G4 (286.7U), which included doses containing chitosan, while it decreased to the lowest level in group G2 (324.8U) which included dosing animals with fructose (Figure 1A- F).

Table (3) shows that ALT, AST, ALP, albumin, SOD and CAT in groups G3 and G4 did not show statistical differences ($P>0.05$) when compared with each other groups or with the control group (G1), while group G2 showed clear differences in those parameters when compared with the other groups ($P<0.05$).

Table 1: Total body weight change over the 4-week trial period

Experimental Groups	Mean ± Standard Deviation		
	Initial Body Weight /g	Final Body Weight/G	Body Weight Gain/g
G1	197.88^{a} ±2.30	237.25^{b} ±6.92	39.37 ±2.72
G2	197.87^{a} ±2.74	215.75^{c} ±3.69	17.87 ±6.02
G3	197.63^{a} ±3.25	235.25^{b} ±8.61	37.62 ±2.55
G4	198^{a} ±2.88	229^{b} ±6.57	31±3.61

***In** a column, means with distinct superscripts indicate significant differences (p <0.05).

Table 2: Liver weight and index over the 4-week experimental period

Experimental Groups	Mean ± Standard Deviation	
	Mean of Liver Weight/g	Liver Index/g
G1	2.29^{a} ± 0.03	0.965^{c}
G2	1.81^{b} ± 0.06	0.839^{e}
G3	2.30^{a} ±0.028	0.978^{c}
G4	2.28^{a} ±0.04	0.996^{c}

*Means with different superscripts within a column indicate significant differences (p<0.05).

Table 3: Evaluation of serum level of studied parameters in experimental groups

Parameters	Experimental Groups (Mean ± Standard Deviation)			
	G1	G2	G3	G4
ALT (U/I)	65.82 ^{a} ± 1.99	100.5^{b} ±1.26	66.95 ^{a} ±2.59	71.27 ^{a} ±3.74
AST (U/I)	125.53 ^{a} ±1.33	172.2 ^{b}±2.66	127.4 ^{a} ±2.49	134.1 ^{a} ±3.54
ALP (U/I)	71.27 ^{a} ±3.74	119.6 ^{b} ±13.43	72.01 ^{a} ±5.31	82.16 ^{a} ±5.38
Albumin(gm/dl)	2.43 ^{a} ± 0.29	1.41 ^{b} ±0.43	2.44 ^{a} ±0.33	2.07 ^{a} ±0.17
SOD (U)	11.99 ^{a} ± 0.88	32.99 ^{b}±1.62	13.54 ^{a} ±0.82	17.92 ^{a} ±2.47
CAT (U)	252.8 ^{a} ±10.33	324.8 ^{b} ±9.26	262.2 ^{a} ±7.35	286.7 ^{a} ±9.45

Significant differences (p <0.05) are indicated by means in a row with distinct superscripts.

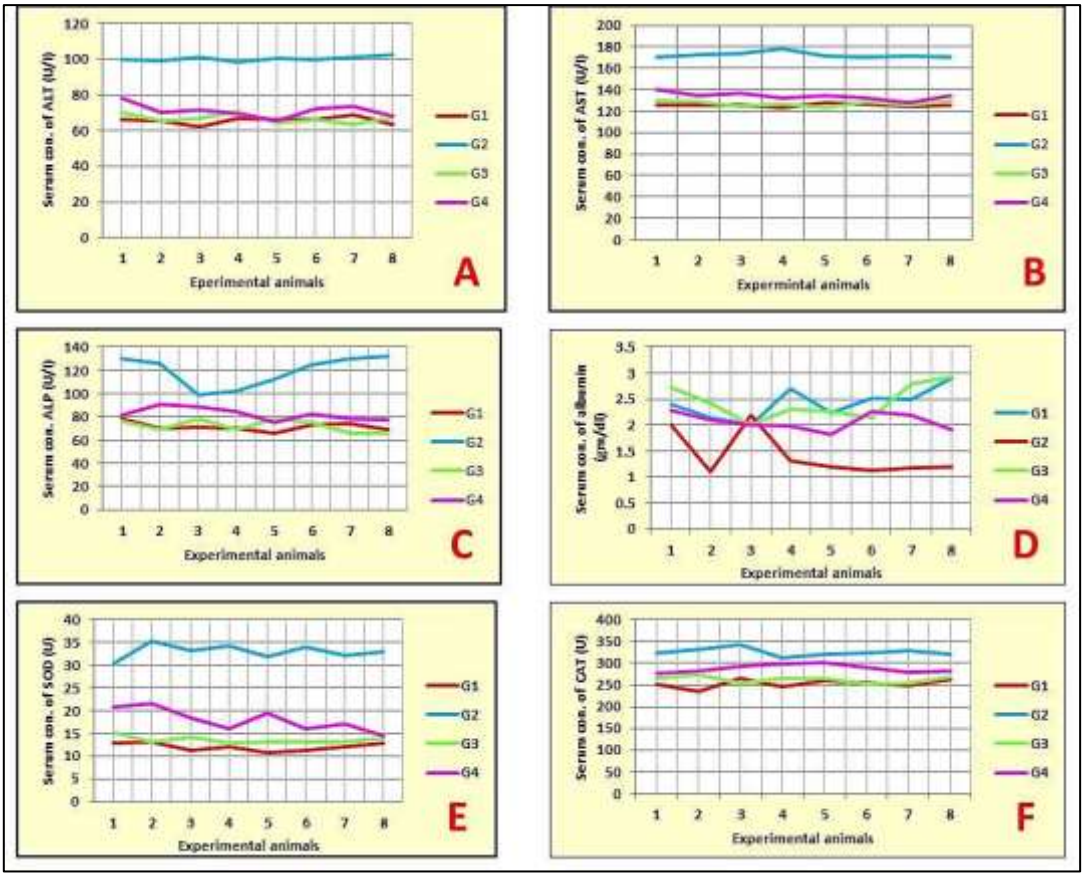

Figure 1: Evaluation liver activities by detection serum level of ALT (A), AST (B), ALP (C), albumin(D), SOD (E) and CAT (F) among experimental groups

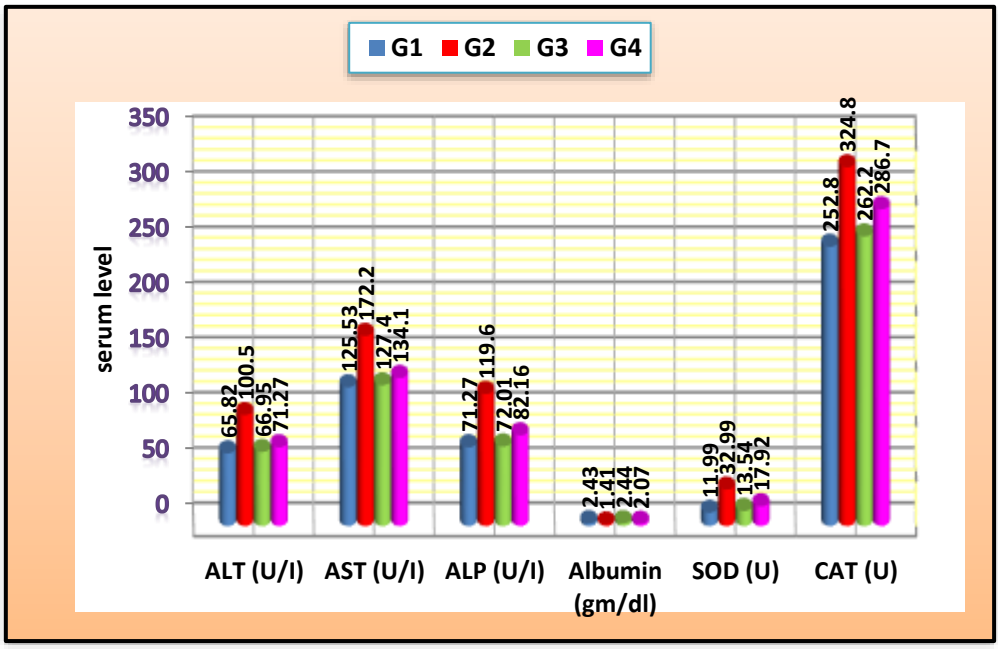

Figure 2: Compared studied parameters among experimental groups

3.2. Histopathological Results

Histopathological examination revealed normal hepatic architecture on the control group's liver slides (Figure 3A). Hepatocyte necrosis and hydropic and fatty changes were observed in sections of the liver from the fructose-induced hepatotoxicity rat group (Figure

3B). Mononuclear cells invaded portal tracts and vascular congestion was observed in hepatic parenchyma. Additionally, bridging fibrosis and fibrosis of the portal tracts and hepatic parenchyma were observed.

Histopathological sections of the livers of rats in the G4 group revealed normal hexagonal hepatocytes with normal central nuclei, a slight enlargement of Kupffer cells, and hepatocytes that displayed fatty and hydropic changes (Figure 3D). In the chitosan-treated group, liver sections also revealed mild mononuclear inflammatory cell infiltrates

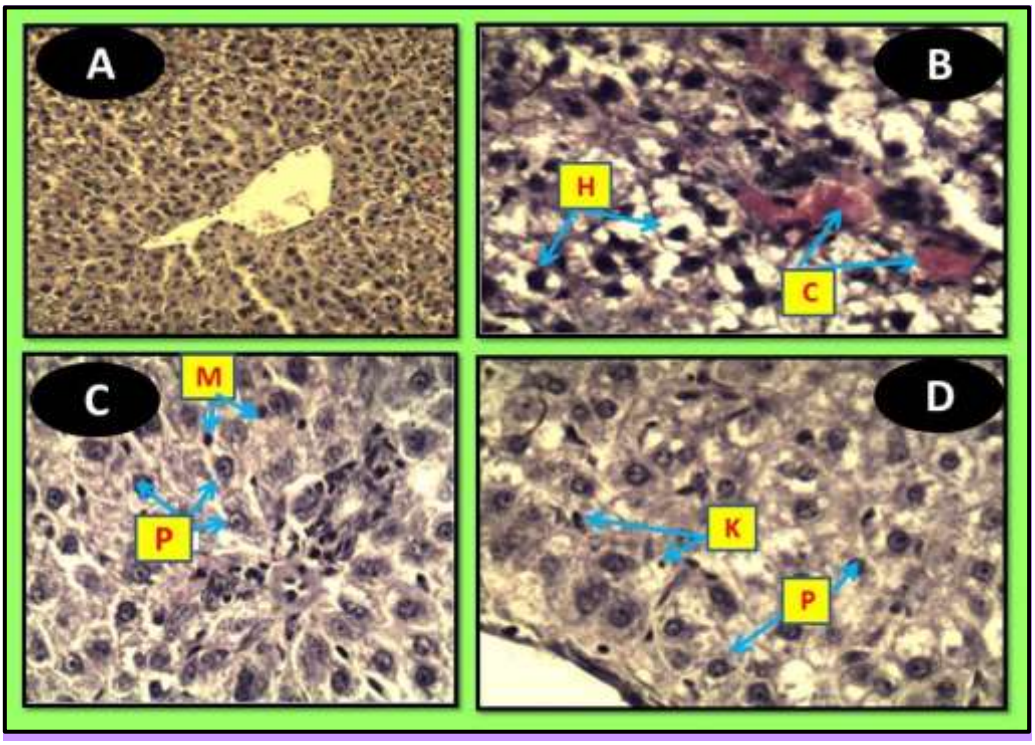

Figure 3: Histopathological section in the liver of experimental groups : A= Histopathological section in the liver of rat in G1(control) group shows normal hepatic lobule (H&E stain 40 X), B= histopathological section in the liver of rat G2 group:: notices hydropic degeneration (**H**) swelling in the hepatocytes with central prominent nuclei , congestion in the bile duct (**C**) (H&E stain 400 X), C= Histopathological section in the liver of rat in G3 group shows: normal hexagonal hepatocytes with normal central nuclei(**P**), few mononuclear mainly lymphocytes in hepatic tissue(**M**) (H&E stain 400 X), D= histopathological section in the liver of rat G4 group: notices normal hexagonal hepatocytes with normal central nuclei(**P**), slight enlargement of Kupffer cells(**K**) (H&E stain 400 X).

4. Discussion

Due to its availability in many types of fruits, vegetables and foods, fructose is one of the most sugars consumed by humans. However, because of its proven negative impact on the body, especially the liver, there is an increase in research on how to reduce its effect or find a successful alternative to reduce its consumption. The current study presented chitosan as a protective factor for the liver from fructose damage and that it can be used as a substitute for carbohydrates to avoid damaging the liver by testing the efficacy of chitosan on fructose fed rats.However, recent research has focused on the potential of many biocompatible natural compounds and biodegradable polymers as drug delivery systems or as regulators to reduce tissue damage from the use of toxic compounds. One of the most widely used monopolymers in the pharmaceutical field is chitosan, which is obtained from deacetylation of chitin

[19],[20]. The protective effect of chitosan on hepatocytes has been investigated by researchers in the last few years, after causing liver injury of laboratory animals with various toxic substances and treating them with chitosan in free form or nanoparticles [21]-[23].Our experience, however, showed that chitosan has hepatoprotective effects, causing improvement in serum albumin, antioxidant defense systems ALT, AST and ALP in an experimental model of toxic hepatitis caused by high concentration of fructose in rats.A previous study showed that the use of chitosan, chitosan vesicles, and ascorbic acid reducesoxidative stress associated with alcoholic hepatotoxicity. The effect of chitosanone in decreasing oxidative stress in rats with alcoholic hepatotoxicity was weaker than that was produced by ascorbic acid under the same experimental conditions [24]. In rats induced hepatotoxicity by repeated administration of carbon tetrachloride, chitosan showed protection by increasing antioxidant activity as well as relieving blood biochemical disturbancesand histological changes that appeared in the liver tissue [21],[22].In the experimental model of toxic hepatopathy caused by acetaminophen in rats, experimental studies have demonstrated that chitosan has hepatoprotective effects due to improved endogenous antioxidant defense systems and reduced lipid peroxidation [18]. In an experimental study conducted on rats, the use of titanium (IV)–dithiophenolate complex chitosan nanocomposites decreased the activity of oxidative stress markers and improved the functional and structural liver injuries caused by carbon tetrachloride [23]. Chitosan's effects on liver tissue regeneration in rats with thioacetamide-induced liver injury have been the subject of additional research [25]. In addition to its curative and proliferative effects, chitosan also acted as an anti-inflammatory agent, lowering TNF- levels in laboratory animals, enhancing hepatic and renal function, and correcting structural changes in thioacetamide-induced liver-damaged animals [25].Chitosan and chitosan nanoparticles have also been shown to protect the liver from damage caused by a variety of hepatotoxic substances, including diethylnitrosamine in rats [26] and emamectin benzoate in mice [27]. In animals with diethylnitrosamine-induced liver toxicity, rats treated with chitosan had lower levels of serum liver marker enzymes and increased activity of antioxidant parameters and serum albumin [26]. The use of chitosan and chitosan nanoparticles changed the biochemical changes in the blood, reduced oxidative stress, decreased the expression of some specific genes in the liver, and improved the structural changes in the hepatocytes [27].

Based on the ion gelation method, other researchers obtained chitosan nanoparticles that protected rats from 2-nitropropane-induced hepatic injury by reducing inflammatory markers, restoring normal liver conformation, and improving functional biochemical parameters and oxidative stress [28]-[29]. Chitosan supplements and the prevention or treatment of iatrogenic hepatotoxicity have been the subject of several studies. A research experiment carried out by Santosh et al. investigated the protection of the liver in rats given the antitubercular medications isoniazid and rifampicin, both of which had hepatic side effects. In rats treated with both substances, chitosan significantly reduced marker enzyme levels and prevented liver changes [30]-[32].

5. Conclusion

Chitosan improves liver problems that are caused by large amounts of fructose by reducing the values of biochemical parameters such as ALT, AST and ALP. At the same time, it increases the antioxidant activity of parameters such as SOD and CAT and restores serum albumin ratio to normal levels. It can be deduced that chitosan may be useful as an antioxidant in cases of hepatotoxicity and has the potential to protect the liver by acting as powerful natural agents. Finally, it was found that chitosan is one of the sugars that has a positive effect on the efficiency of the liver. It may be one of the best options when used in food products.

6. Recommendations

Further molecular studies verify the effects of chitosan on other body tissues such as the kidneys, reproductive system, blood and others.

7. Conflict of Interest

No known conflicts of interest exist.

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