MENOUFIA JOURNAL OF AGRICULTURAL BIOTECHNOLOGY

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HEPATOPROTECTIVE EFFECTS OF CHITOSAN AND CHITOSAN NANOPARTICLES

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Received: Sep. 4 , 2022 **Accepted: Sep. 24, 2022**

ABSTRACT: According to reports, when exposed to hazardous compounds like ethanol, hepatocytes are vulnerable to the harmful effects of oxidants. Chitosan is a potential chemical for the field of toxicity prevention because it possesses a variety of therapeutic qualities.

The chemical makeup of chitosan and chitosan nanoparticles were assessed in this study using FTIR spectroscopy, and their particle size and antioxidant activity were assessed in vitro using DPPH. The in vivo hepatoprotective properties against ethanol-induced liver injury were assessed in male Wister rats. In both investigations, silimarin (100 mg/kg b.w.) and chitosan plus chitosan nanoparticles (200 mg/kg b.w.) were given orally. 3.76 gm/kg b.w. of 40% ethanol administered orally for 30 days caused liver damage. In the DPPH scavenging activity assay, chitosan and chitosan nanoparticles both showed antioxidant activity; however, chitosan nanoparticles were more effective than chitosan overall. Hepatotoxicity was evaluated by measuring plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, and plasma antioxidant status (MDA concentration, and catalase activity). Raised plasma AST, ALT, and MDA levels in rats after ethanol treatment showed liver injury. In a preventive model, the administration of chitosan, chitosan nanoparticles, and silimarin reduced the harmful effects of ethanol on the aforementioned plasma parameters. According to the findings of the current investigation, chitosan and chitosan nanoparticles significantly inhibit ethanol-induced liver damage and have antioxidant and hepatoprotective properties.

Key words: Chitosan ; nanoparticles ; Hepatoprotective

INTRODUCTION

Chitin, a crucial component of the shells of crustaceans including crab, shrimp, and crawfish, is converted into the natural carbohydrate biopolymer chitosan through a process known as deacetylation (DA). Chitin is the second most prevalent natural biopolymer found in nature after cellulose (No et. al., 1989) Chitosan is a fibre resembling cellulose, but unlike plant fibre, it has special qualities like the capacity to create films, optical structural characteristics, and much more. Additionally, chitosan has a positive ionic charge that allows it to chemically bond with negatively charged lipids and fats, which helps bile acids (Sandrof, 1991). A non-toxic, biodegradable, and biocompatible polymer is chitosan. The last few years have seen an increase in interest in chitin polymers, particularly chitosan, as one of the promising renewable polymeric materials due to its wide range of applications in the pharmaceutical and biomedical fields for enzyme immobilisation and purification, in chemical plants for wastewater treatment, and in the food sectors for food formulations as binding. gelling, thickening, and stabilising agent (Knorr, 1984).

Numerous applications have made use of nanoparticles (NPs) made from synthetic or natural polymers. A nanoparticle's potential applications depend on a number of elements, including the kind of substance, particle form, and concentration. The size, content, crystallinity, and shape of NPs all play a major role in determining their intrinsic qualities. Applications for these compounds are determined by their chemical composition, size, shape, charge, hydrophobicity, and surface characteristics such as the presence or absence of functional groups or other chemical compounds (Zhao et al. 2011). Indeed, due to its biodegradability and biocompatibility, chitosan

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has been investigated as a material of choice to create NPs in recent years.

Chitosan nanoparticles (CSNP) may have a wide range of novel application possibilities due to the special properties of NPs, such as their small size and quantum size effect. (Gouda *et al.*, 2014). This study sought to determine whether chitosan and chitosan nanoparticles made in the lab from shrimp waste had any anti-oxidative or protective effects on hepatocytes.

MATERIALS AND METHODS

1- Materials:

1.1 Crude shrimp shells:

The local market provided some dried shrimp shell trash, which was then cleansed of debris, sand, and salt crystals under the defined conditions outlined by (Sorgeloos 1986). Shells were cleaned multiple times in fresh water, dried overnight at 60 °C in a forced air oven, ground into powder with a mill, and then stored at 0 °C for whatever long they were required.

1.2 Reagents:

Sodium hypochlorite, glucose, glacial acetic acid, hydrochloric acid, sodium hydroxide, acetone, ascorbic acid, and sodium tripolyphosphate (STPP) were purchased from El-Nasr Pharmaceutical Chemicals, El-Ameriea, Cairo, Egypt. Diphenyl-1-picrylhydrazyl (DPPH) was purchased from ROTH Bestellen sie zum (Nulltarif, Germany). Sulphoric acid was obtained from Merck, Darmstadt, Germany, and other kits were purchased from El-Gomhorea company for Pharmaceutical Chemicals.

2- Methods:

2.1 Preparation of chitosan sample:

Four steps were used in the preparation process: demeneralization (DM), which involved treating the raw material with 0.7 N HCI at room temperature for 15 min; deproteinization (DP), which involved treating the material with 1.2 N NaOH for 2.5 hr at 70–75 °C; decoloration (DC), which involved soaking in acetone for 10 min and drying for 2 hr under a hood; bleaching with 0.32% sodium hypochlorite solution for 15 min at The material was removed following each procedure, rinsed with distilled water to a pH of 7, and then dried in an oven at 60 oC overnight (Andreas and Bernd, 2004)

2.2 Preparation of chitosan nanoparticles:

In a nutshell, 400 cc of a 10% ascorbic acid aqueous solution with 1% of glucose was continuously stirred at room temperature to dissolve 1 g of chitosan. After the chitosan in the ascorbic acid had completely dissolved, the STPP (0.2 gm in 100 ml distilled water) was added drop by drop while stirring consistently, waiting until a milky white suspension appeared. After vigorously swirling the solution for 30 minutes, it was centrifuged at 10,000 rpm for 30 minutes to generate a pellet, which was then cleaned with distilled water. Before being employed, the nanoparticles were lyophilized and kept at 4 ° C. (Megha, 2018).

2.3 Characterization of chitosan and chitosan nanoparticles :

2.3.1 FTIR Spectroscopy

The NICOLET IS 10 instrument was used to carry out the Fourier transform infrared (FT-IR) spectrum studies. Potassium bromide was evenly mixed with the samples, and the resulting discs were scanned at a scanning speed of 400 to 4000 cm-1. (Sebastian et al., 2018)

2.3.2 Evaluation of particle size

A particle size analyzer with laser diffraction, the LS Beckmen Coulter LS 13320, was used to measure the particle size distribution at room temperature. Samples of nano-chitosan were dissolved in dispersants (distilled water, pH 7), then put in a 3 ml cuvette for analysis and fractionation using the visible light scattering principle. (Hui, 2018).

2.4 Antioxidant activity of chitosan and chitosan nanoparticles :

Effect of scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals :

A 1,1-diphenyl-2-picrylhydrazyl was used to test the free radical scavenging capacity (DPPH). With some modifications, the approach employed in this study was adapted from work by (Brand-Williams *et al.*, 1995). In a 10 ml test tube, 0.2 ml of MeOH and 0.3 ml of various sample concentrations (ranging from 25 to 200 g ml^{-1}) dissolved in MeOH were combined. 3.0 ml of DPPH (2.5 ml of 75 M in MeOH) was then added to get the final volume. After 30 minutes at room temperature, the solution was tested to determine its absorbance at 517 nm (A 517). Following is how the DPPH scavenging effect was determined:

Scavening effect % = [A_0 – (A – A_b) / A_0] x 100

Where:

A_0 = A_{517} of DPPH without sample
A = A_{517} of sample and DPPH
A_b = A_{517} of sample without DPPH

2.5 Invivo study for test the protective effect of chitosan and chitosan nanoparticles against ethanol administration:

The Research Institute of Ophthalmology in Giza, Egypt provided the rats. Additionally, the work was done at the animal home. Twenty male albino rats (weighing between 90 and 110 g) were used in this study to examine the protective effects of chitosan and chitosan nanoparticle oral administration. As an adjustment period, the rats were given a basic diet (BD) and water ad libitum for 15 days. They were separated into five groups and kept in individual stainless steel cages. The BD was served to all groups. Daily diet intake was tracked.

As controls, the first group (group A) was given tape water to drink. The other four groups were given tape water and 40% V/V ethanol by stomach tube every day for 30 days at a dose of 3.76 gm/Kg body weight. The second group (the ethanol group) receives no additional care. The fourth group (ethanol + chitosan nano group) was simultaneously treated by stomach tube with chitosan nano (200 mg / Kg body weight), and the final group (ethanol + silymarin group) was treated with silymarin drug (100 mg / Kg body weight). The third group (ethanol + chitosan normal group) received chitosan normal (200 mg / Kg body weight). Prior to taking blood samples, all rats fasted. The blood was taken from the ocular plexuses, after 30 days, the rats were anesthetized using diethyl ether.

Blood sampling: Blood samples were received from orbital plexus by means of a fine capillary glass tube according to (Schalm, 1986). Each sample was placed in a dry clean centrifuge tube contained heparin as anti-coagulant; the blood was centrifuged for 10 minutes at 3000 rpm to separate the plasma from cells. The clean non-haemolyzed supernatant plasma was then piptte into epindorff tube and kept frozen until analysis. The both of aspartate amino transaminase (AST) and alanine amino transaminase (ALT) activities were measured in plasma according to the method described by (Young, 1990), the total protein was determined according to the method described by (Doumas *et al.*, 1971), lipid peroxidation in plasma was monitored by determining the concentration of malondialdehyde (MDA) as described by (Ohkawa *et al.*, 1979) and catalase activity was determined in plasma as described by (Aebi 1984).

2.7 Statistical analysis

The Statistical Package for Social Sciences was used to do statistical analysis on all data given as mean SE (SPSS 18.0 software and Microsoft Excel 2010). For testing, one-way analysis of variance (ANOVA) with repeated measures was used to analyse group differences, followed by post-hoc comparisons (LSD test). At P 0.05, differences were deemed statistically significant. (Landue and Everitt, 2004).

RESULTS AND DISCUSSION:

Characterization of chitosan and chitosan nanoparticles :

FTIR Spectroscopy of chitosan and chitosan nanoparticles :

The produced chitosan samples' FTIR spectra were displayed in Figs. 1 and 2, displaying the

hydroxyl and amine group-specific absorption bands at 3438 and 3290 cm1. The alkyl chains are what cause the absorption bands to form between 2920 and 2883 cm1. The areas at 1650 cm1 and 1380 cm1 are where the amide carbonyl bands may be seen. The C-O bond is represented by prominent bands between 1075 and 1092 cm1, which are the distinctive peaks for polysaccharides. Band at 1373 cm-1 displays the stretching vibration of C-H from CH2OH groups, while bands at 1072 cm-1 and 983 cm-1 display the stretching vibration of C-O from CH-OH, respectively. These results are consistent with (Nie *et al.*, 2015; Yasmeen *et al.*, 2016).

The FTIR spectrum of CS-TPP nanoparticles is similar to that of the chitosan sample, but there is a difference at 3421 cm1 where the spectrum is broadened as a result of the interaction of the phosphate group from TPP with the NH2 group from chitosan; 1539 cm1 demonstrates a difference in the N-H group; and there is also a difference in the absorption bands at 1076 - 902 cm1 due to (Jafary *et al.*, 2016; Nie *et al.*, 2015).

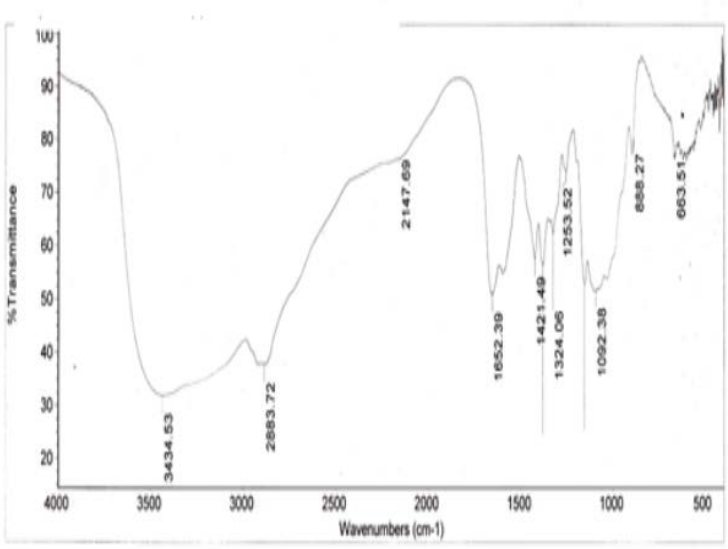

Figure 1 : FTIR of chitosan

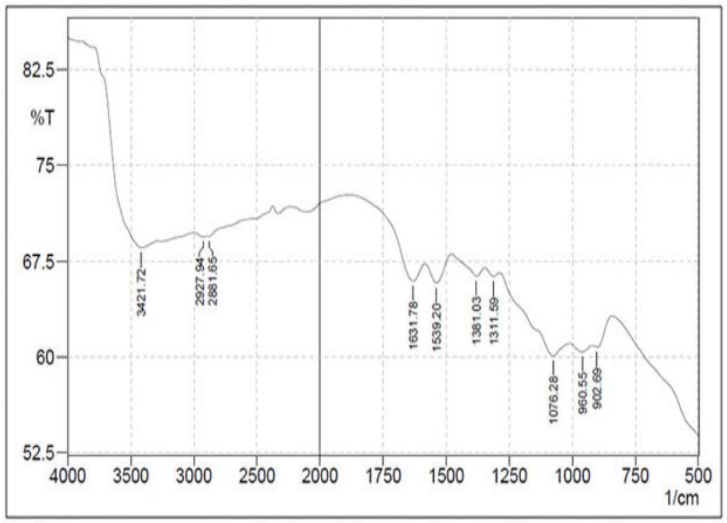

Figure 2: FTIR of chitosan nanoparticles

Particle size of chitosan nanoparticles:

The Dynamic Light Scattering (DLS) approach, which measures particles based on diffusion of Brownian motion—or the random movement of particles in a liquid media as a result of collisions with solvent particles—can analyse the size of nanoparticles. According to measurements, the generated chitosan nanoparticles were 1.03 nm or 1033 nm in size, as illustrated in Figure (3). According to (Mohanraj and Chen 2006), solid particles with a size range of 10-1000 nm are what are known as nanoparticles. It has been suggested that size reduction utilising a magnetic stirrer at high speed can evenly disperse energy throughout the solution, resulting in a more homogeneous size. TPP may increase the chitosan matrix bond while decreasing the size of nanoparticles. (Du et al., 2009).

Antioxidant activity of chitosan and chitosan nanoparticles:

The DPPH is a stable free radical that is used to estimate the capacity of antioxidants to scavenge free radicals. By turning DPPH's purple colour into a yellow (a, a-biphenyl-b-picrylhydrazine) solution, which can be measured by measuring the absorbance at 517 nm, the antioxidant power of the substance was demonstrated (Belmekki and Bendimerad, 2012). In fact, the decrease of alcoholic DPPH solutions is demonstrated by the free radical scavenging method (DPPH) in the presence of an antioxidant that donates hydrogen (Koleva et al. 2002). Therefore, the degree of decolorization determines the chitosan and chitosan nanoparticles' capacity for scavenging. The potentials of the produced chitosan and chitosan nanoparticles to scavenge DPPH radicals are clearly shown in Fig. 4. Both chitosan and chitosan nanoparticles demonstrated this ability. However, chitosan's activity was less than chitosan nanoparticles'. The ability of antioxidants to donate hydrogen atoms was thought to be the cause of their impact on DPPH scavenging (Zhang et al., 2013). Therefore, the significant hydrogen-donating ability of the chitosan samples may be responsible for their ability to scavenge DPPH.

Previous research demonstrates that the amount of active hydroxyl and amino groups in the polymer chains was connected to the antioxidant activity of chitosan (Feng *et al.*, 2008). High deacetylated chitosan has more amino groups, which boosts the antioxidant action. On a per-unit-mass basis, it is hypothesised that polysaccharides with smaller MW would have more reductive hydroxyl groups to scavenge free radicals (Liu *et al.*, 2010). Therefore, the fact that its MW is smaller than the chitosan sample in our investigation should explain the better DPPH scavenging ability displayed by chitosan nanoparticles.

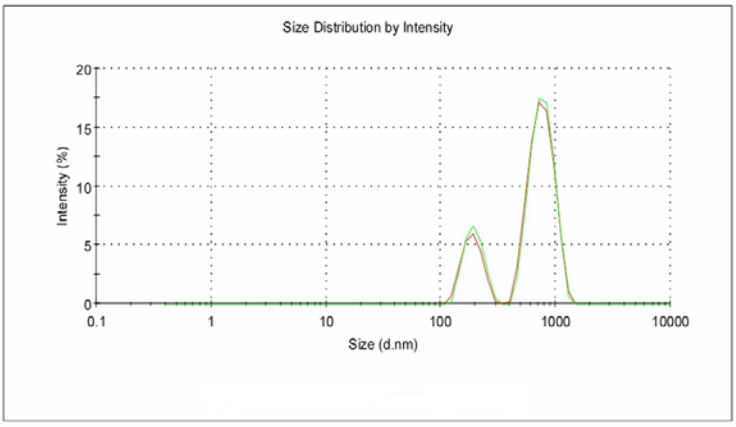

Figure 3: Particle size of chitosan nanoparticle

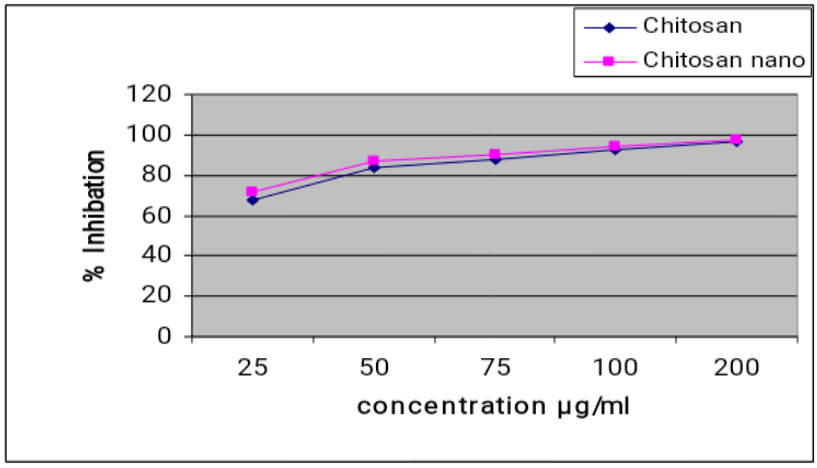

Figure 4: Free radical scavenging of chitosan and chitosan nanoparticles

Biological effects of chitosan and chitosan nanoparticle :

Liver functions:

The results are shown in Table 1 and represent the average values across the entire time period. The aspartic transaminase (AST) and alanine transaminase (ALT) levels of albino rats were 34.75 and 28.25 IU/L and 158.24 and 85.5 IU/L, respectively, in the positive group after receiving ethanol treatment. In the negative group, albino rats were fed a regular diet. The enzyme liver marker shows a decrease in AST (141.25, 142.25, and 127) and ALT (69.75, 72.75, and 62.75) in rats treated with chitosan, nano-chitosan, and silimarine in addition to being given ethanol. These findings are quite similar to those that were supported by a study by. Michael et al., (2005).

The seum level of AST and ALT are illustrated in Table 1 in which can be found that the level of liver function parameters in ethanol treatment moder groups are increased singnificantly as compared to the normal groups, the toxic effect of ethanol may be attributed to hebatic injury as a result of hepatic injury, however, these increases were attenuated by chitosan and nanochitosan.

The plasma total protein concentration in the ethanol-treated group was significantly lower (P 0.5) than in the control group, with mean values of 6.175 and 7.125 mg/dl, respectively. However, compared to the ethanol group, the chitosan and silymarin groups significantly increased (P 0.05) the mean value of total protein. It is well established that chronic ethanol administration has a significant impact on how lipids and lipoproteins are metabolised. Additionally, this leads to the buildup of hepatic lipids and lipid peroxides, which disturb the equilibrium between the levels of pro-oxidants and antioxidants, causing the hepatic cells to autooxidize. (Zhou et. al. 2003). As a result, the hepatic cells experience oxidative stress, which is the most obvious early sign of alcohol-induced liver damage. The cytosolic enzymes leak into the blood stream when the liver cell membrane is damaged (Ramaiah 2007). Therefore, an essential quantitative indicator of the severity of liver injury is the rise of these cytosolic enzymes in the blood stream. This study looked into the hepatocellular oxidative damage caused by ethanol and how chitosan and nano-chitosan protect against it.

Oxitative stress and antioxidant markers

Malondialdehyde (MDA) levels and catalase activity:

Oxidative stress in cells is a condition of imbalance due to excess formation of reactive oxygen species and decreased activity of antioxidant defense systems. The levels of ROS

are regulated by a variety of cellular defense mechanisms consisting of enzymatic and non-enzymatic systems, (Kim et al., 2003).

Results are given in Table 2 represented the mean values through the whole period. Data indicated that malondialdehyde (MDA) concentration and catalase (CAT) activity were 11.32 mg/dl and 697.72 IU/L respectively for negative control group which feeding on standard diet, where group treated with ethanol (positive control group) revealed significantly increased malondialdehyde (MDA) 19.55 mg/dl and significantly decreased catalase (CAT) activity 535.92 IU/L. In rats subjected to ethanol and treated with chitosan, nano-chitosan and silimarine, the antioxidant markers indicate a decrease significantly of malondialdehyde (MDA) level (13.3, 11.52 and 11.4 mg/dl) respectively and increase significantly of catalase (CAT) activity (573.72, 606.35 and 654.35 IU/L) respectively when compared with positive control.

The increase in MDA levels, an end product of endogenous lipid peroxidation confirms that lipid peroxidation is believed to be involved in several disease states, such as diabetes (Baynes, 1991).

Table 1: Effect of chitosan and nano-chitosan on AST, ALT and total protein activities in rats treated with ethanol for 30 days.

Groups	AST IU/L	ALT IU/L	Total protein g/dl
(-) Control	34.75±3.4 ^{a}	28.25±2.5 ^{a}	7.125±0.53 ^{b}
(+) Control	158.25±4.2 ^{c}	85.50±2.3 ^{c}	6.175±0.25 ^{a}
Chitosan	141.25±2.5 ^{bc}	69.75±3.1 ^{bc}	6.600±0.4 ^{ab}
Nano-chitosan	142.25±2.2 ^{bc}	72.75±2.4 ^{bc}	6.225±0.22 ^{a}
Silymarine	127.00±1.8 ^{b}	62.75±2.5 ^{b}	6.625±0.45 ^{ab}

Each value is the mean ± SD. Means have different superscript letters indicate significant variation at ($P \leq 0.05$), while the same letters indicate non significant variation.

Table 2: Effect of chitosan and nano-chitosan on malondialdehyde (MDA) levels and catalase (CAT) activity

Groups	CAT U/L	MDA mmol/l
(-) Control	697.72±3.8 ^{d}	11.32±1.2 ^{a}
(+) Control	535.92±4.3 ^{a}	19.55±1.4 ^{c}
Chitosan	573.72±3.6 ^{ab}	13.30±1.4 ^{b}
Chitosan nano	606.35±3.2 ^{b}	11.52±0.7 ^{ab}
Silymarine	654.35±2.6 ^{c}	11.40±0.7 ^{ab}

Each value is the mean ± SD. Means have different superscript letters indicate significant variation at ($P \leq 0.05$), while the same letters indicate non significant variation.

Conclusion

This study was designed to answer the question of whether there is a protective effect of chitosan and nanochitosan on liver cells in ethanol-treated rats. It can be said from the obtained results that both chitosan and nanochitosan showed a positive effect as substances that can protect liver cells from damage by ethanol by showing a clear improvement in indicators of liver function in the blood and also showed a positive effect on the tested oxidation state indicators (MDA and catalase).

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التأثيرات الوقائية الكبدية للشيتوزان والنانو شيتوزان

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الملخص العربى

إن خلايا الكبد تتعرض للتأثيرات الضارة للمواد المؤكسدة عند تعرضها لمواد سامة مثل الإيثانول.وقد اوضحت نتائج الابحاث في كثير من المراجع العلمية فعالية جزيئات النانو شيتوزان والشيتوزان ضد هذا التاثير الضار. وقد امكن عن طريق التحليل الطيفي FTIR تحدد حجم جسيمات النانوشيتوزان والشيتوزان .مما يساعد في تقيم النشاط المضاد للميكروبات لعينات الشيتوزان, ونشاطها كمضادات الأكسدة .وتم تقييم وتحديد التأثيرات المرضيه للكبد في ذكور الفئران متمثلا في تلف الكبد الناجم عن الإيثانول في النماذج الوقائية والعلاجية, حيث استخدمت جزيئات النانو شيتوزان والشيتوزان (٢٠٠ ملجم / كجم من وزن الجسم) ، والسيليمارين (١٠٠ ملجم / كجم من وزن الجسم) عن طريق الفم الي المعدة مباشرة.

وتم تحفيز إصابة الكبد باستخدام تركيز ٤٠ ٪ من الإيثانول (٣,٧٦ جم / كجم من وزن الجسم ، عن طريق الفم) لمدة ٣٠ يومًا ، وقد اوضحت النتائج عند استخدام جزيئات النانو الشيتوزان والشيتوزان نشاطًا مضادًا للأكسدة في اختبار مقاومه الشقوق الحره DPPH ، بينما كانت جزيئات الشيتوزان النانوية أكثر فعالية مقارنة بالشيتوزان العادي في تحسين مستويات ال((ALT،AST)) ، ، البروتين الكلي و(MDA) و الكاتاليز وتسبب تناول الإيثانول في تلف كبدي شديد في الفئران كما يتضح من ارتفاع مستويات البلازما AST و ALT ومحتوى MDA.تناول الشيتوزان النانو شيتوزان والسيليمارين منع التأثير السام للإيثانول على معلمات البلازما المذكورة أعلاه في النموذج الوقائي. خلصت الدراسة الحالية إلى أن الجسيمات النانوية للشيتوزان والشيتوزان لها نشاط هام مضاد للأكسدة ووقائي للكبد ضد السمية الكبدية التي يسببها الإيثانول.