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Original Research Article

Physiological Effects of Chitosan Nanoparticles (CS-NPs) Against Bisphenol a Induced Toxicity in Male Albino Rats

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Abstract: The purpose of this study was to ascertain how oral administration of 30% and 40% chitosan nanoparticles on bisphenol-A affected the lipid profile, renal function, and liver enzyme parameters (ALT, AST, and ALP) in white male rats. The liver enzyme markers AST, ALT, ALP, urea, creatinine, triglycerides TG, cholesterol TC, low-density lipoprotein LDL, and very-low-density lipoprotein "vLDL" were all significantly elevated ($p < 0.05$) by bisphenol-A, while the concentration of high-density lipoprotein HDL decreased. While a significant decrease was observed in the parameters of liver enzymes, urea, creatinine, triglycerides (TG), cholesterol (TC), low-density lipoprotein (LDL), and very-low-density lipoprotein (vLDL) and an increase in the value of high-density lipoprotein (HDL) when treated with chitosan nanoparticles.

Keywords: Chitosan Nanoparticles, Bisphenol A, Renal Function, Liver Enzymes, Lipid Profile.

INTRODUCTION

Nanoparticles are defined as particles with sizes ranging from one to one hundred nanometers. They differ from their counterparts in raw materials in that they have a high surface-to-volume ratio and other novel physical and chemical characteristics like color, solubility, strength, diffusion, magnetism, and optics. A variety of techniques, including chemical, physical, and biological ones, can be used to produce nanoparticles. As antimicrobial agents, nanoparticles are very effective at preventing the growth of resistant microbes of all kinds.

It is distinguished by its reduced toxicity and resistance to heat [1]. In addition to Nano-chitosan, which is characterized by the body's daily requirement for it to complete various metabolic processes in it, chitosan is an organic nanoparticle that is known for its capacity to inhibit microbes and for having no adverse side effects that may arise from its use [2]. Due to their biological and mucosal-resonant properties, chitosan nanoparticles can increase the permeability of the mucous membrane. They are natural materials with physical, chemical, antimicrobial, and biological properties that make them environmentally friendly and have biological activity that does not harm humans. They are also a proven antioxidant agent that can scavenge free radicals and chelated metal ions by donating hydrogen or a single pair of electrons. Thus, it enhances the transport through the cell-to-cell pathway of nanoparticles and can induce structural reorganization-localization of tight junction-associated proteins [3].

The organic substance bisphenol A (BPA) is utilized in the production of PVC plastics, epoxy resins, and polycarbonate. It was originally manufactured in 1891, but it wasn't until scientists developed polycarbonate plastic in the 1930s using bisphenol A that it became extensively used. Paints, dairy packaging, primers, milk bottles, water bottles, infant formula bottles, and plastic plates are all made with epoxy resins and polycarbonate plastics. BPA in plastics migrates

from the packaging material into the food in the container [4]. Heating, misuse, contact with alkaline or acidic substances, and exposure to microwaves, which causes BPA to mix with the food.

Numerous investigations have verified that unconjugated BPA is transferred to the serum of adults and fetuses through food interaction. Since many people are exposed to extremely small amounts of BPA and it is hard to detect for extended periods of time, its entrance into food is thought to be the most hazardous [5].

Glasses, food packaging, reusable food storage containers, plastic cutlery, and thermal paper all contain BPA. According to [6], BPA can enter the body by food, skin contact, or inhalation when these products are released into the environment. BPA has a close relationship with chronic renal disease [7]. Because BPA-containing materials are used in the dialysis process, a prior study found that adult patients receiving continuous hemodialysis had BPA values six times greater than healthy adults. The purpose of this study was to lessen the toxicity that BPA causes in male albino rats by using varying amounts of chitosan nanoparticles.

MATERIALS AND METHODS

Laboratory Animals

This experiment was conducted in the animal house of the College of Veterinary Medicine and the laboratories of the College of Agriculture / Tikrit University with the aim of knowing the effect of chitosan nanoparticles on laboratory animals in which bisphenol was introduced. To accomplish the experiment, 20 healthy adult male white rats were used and it was confirmed that they were not infected with any disease by the veterinarian, which were obtained from the College of Veterinary Medicine / Tikrit University. They are of the Albino Sprague- Dawleyweanling type, 9-10 weeks old and their weights ranged from 210-225 grams. They were randomly divided into 4 groups, each group containing 5 animals, which included the following:

1) The first group ((M1: The negative control group (healthy) which was given standard food and drinking water only.
2) The second group ((M2: The infected group which was given 2 ml Bisphenol A at a concentration of 10 mg/kg animal/day.
3) The third group ((M3: The infected group which was given 2 ml Bisphenol A at a concentration of 10 mg/kg animal/day + 2 ml CS-NPs at a concentration of 30%.
4) The fourth group ((M4: The infected group which was given 2 ml Bisphenol A at a concentration of 10 mg/kg animal/day + 2 ml CS-NPs at a concentration of 40%. The purpose of using two concentrations is to know which one is more effective.

METHODOLOGY

The components mentioned above were mixed with sterile water and then administered to the experimental animals [8]. Once they have been dissolved in the concentrations listed in the groups above. The initial weight was obtained after one day of feeding each laboratory animal separately. The temperature was maintained at 20–25 °C, the lighting was on for at least 12 hours each day, and the food was served in an open manner, prepared in accordance with the guidelines stated by the [9]. During the specified period of the experiment, which lasted for 28 days, immediately after the end of the experiment period, the laboratory animals were fasted for 20 hours and anesthetized using chloroform, blood was drawn directly from the heart [10]. To conduct the necessary tests, approximately 3-5 ml of blood was drawn into the blood collection tubes containing the gel and centrifuged at a speed of 3000 rpm for 15 minutes to obtain the serum, which was kept at the appropriate temperature - 20 °C until laboratory tests were conducted.

Biochemical Examinations

Alkaline phosphatase (ALP) was assessed using standard kits made by the German business ROCHE, while liver enzymes Alanine amino transferase (ALT) and Aspartate amino transferase (AST) were assessed using standard kits made by the British company RANDOX. The Reflotron device was used to read the results in a Standard solution kits (kit) prepared by Biomahreb Company (Tunisia) were used for the purpose of measuring urea and creatinine. These analyses were performed using a spectrophotometer. Total cholesterol, triglycerides and high density lipoprotein (HDL) were estimated as in [11]. Cordance with the supplier's suggested instructions. These analyses were conducted using a Shimadzu spectrophotometer (Japan) at the wavelengths recommended by the manufacturer for each analysis. Concentrations were calculated using equations provided in the manufacturer's instructions. The estimation of vLDL in blood serum was performed as described in [10].

The Statistical Analysis

The statistical analysis of the data was conducted using the experimental system within the Statistical Analysis System [12]. A Completely Randomized Design (CRD) was employed, and means were compared according to Duncan's Multiple Range Test [13], to determine the significance of differences between the means of the factors affecting the studied traits at a significance level of ($P \leq 0.05$).

RESULTS AND DISCUSSION

Effect of Oral Administration of Chitosan Nano CS-NPs on Liver Enzyme Activity in Rats

The impact of oral nano-chitosan treatment on liver enzyme levels in rat groups given bisphenol A for 28 days is shown in Table (1). According to the data, the bisphenol A-treated rat group (M2) had significantly higher liver enzyme activity ($P \leq 0.05$), with values of 103.2, 96, and 176 IU/L, respectively, than the healthy control group (M1), which had values of 81.27, 67, and 147 IU/L, respectively. The results also demonstrated a significant decrease in liver enzyme activity when nano-chitosan particles were added at the above concentrations along with bisphenol A, compared to the M2 group treated with bisphenol A alone.

Table 1: Effect of oral administration of CS-NPs on liver enzyme activity (IU/L) after 28 days

Treatments	AST	ALT	ALP
M1	0.05 ± 81.27 b	0.5 ± 67 c	0.5 ±147 b
M2	0.05 ± 103.32 a	0.5 ± 96 a	±176 a 0.5
M3	0.05 ± 69.86 d	0.5 ± 75.18 b	0.6 ±131.15 d
M4	0.05 ±78.54 c	0.5 ± 66.33 d	0.5 ±145.61 c

Different letters in the same column indicate significant differences at a significance level ($p \leq 0.05$) between the treatments.

M1: Control ،M2: Bisphenol-A

M3: CS-NPs 30 % ،M4: CS-NPs 40 %.

The metabolism of lipids and lipoproteins is known to be significantly and clearly impacted by the consumption of hazardous chemicals. Moreover, it results in the buildup of lipid peroxides and hepatic fat, which throws off the balance of antioxidants and causes hepatocytes to spontaneously oxidize [14]. Consequently, hepatocytes are subjected to oxidative stress, a blatant indication of toxic liver injury.

Enzymes leak into the bloodstream when the liver cell membrane is damaged. Therefore, the elevation of these enzymes in the bloodstream is considered the primary quantitative indicator of severe liver injury [15]. These results are consistent with what [16], found that the use of chitosan nanoparticles has a clear effect in causing a significant decrease in the levels of liver enzymes AST, ALT and ALP.

Nano-chitosan exhibits unique pharmacokinetic properties as a cationic drug. Low molecular weight nano-chitosan shows significant performance in absorbing toxic substances in the bloodstream and gradually reducing their concentration in the body. It is believed that the molecular weight of chitosan plays a crucial role, as low molecular weight chitosan demonstrates high solubility. Chitosan is considered a highly efficient bio-adsorbent for metals due to the presence of amine (-NH2) and hydroxyl (-OH) groups [17].

Effect of Oral Administration of CS-NPs on Kidney Functions Tests in Rats

Table (2) presents the effect of oral administration of chitosan nanoparticles on kidney function in rat groups exposed to bisphenol A for 28 days. The findings revealed a significant increase ($P \leq 0.05$) in creatinine and urea levels in the bisphenol A-treated group (M2), reaching 1.7 and 41.45 g/dL, respectively, compared to the healthy control group (M1), which recorded values of 0.9 and 22.12 g/dL, respectively. The results also showed a significant decrease in kidney function values when adding chitosan nanoparticles at the above concentrations with bisphenol A compared to group M2 to which bisphenol A was added alone.

Table 2: Effect of oral administration of chitosan nanoparticles CS-NPs on kidney function (g/dL) in rats after 28 days

Treatments	critinin	Urea
M1	0.05±0.9 d	0.05 ±22.12 d
M2	0.05±1.7 a	0.5 ±41.45a
M3	0.05±1.05 b	0.05±27.91 b
M4	0.05±1.02 c	0.5±23.08 c

Different letters in the same column indicate significant differences at a significance level ($p \leq 0.05$) between the treatments.

M1: Control ،M2: Bisphenol-A

M3: CS-NPs 30 % ،M4: CS-NPs 40 %.

The toxicity of bisphenol A, which has a noticeable influence on the kidney's capacity to remove the toxicity of this chemical through metabolism, may be the cause of the notable improvement in kidney function observed in laboratory animals after oral administration of BPA. The cause of the rise in urea levels can be explained by the increase in protein

breakdown and the removal of the amine group from amino acids. These harmful substances raise blood urea, which is linked to aberrant renal molecule filtration and a rise in serum creatinine [18].

These results align with the findings of [19], who reported that nano-chitosan particles significantly reduce kidney function markers. Nano-chitosan enhances kidney function by mitigating *oxidative* stress and reducing inflammatory responses in laboratory animals with induced conditions. The substantial decline in renal function was ascribed to the chitosan nanoparticles' capacity to control oxidative stress and inflammatory cytokines, as well as their activity in inhibiting the caspase-3 enzyme, which produced anti-inflammatory and antioxidant effects. Consequently, a nontherapeutic approach to lessen kidney toxicity may be offered by chitosan nanoparticles.

Effect of Oral Administration of (CS-NPs) on Lipid Profile in Rats

Table (3) shows the effect of oral administration of nano-chitosan particles on the lipid profile of rat groups treated with bisphenol A for 28 days. The results indicated a significant increase ($P \leq 0.05$) in the levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), and very-low-density lipoprotein (vLDL) in the bisphenol-treated group (M2), with values of 172.03, 183.21, 119.40, and 34.40 mg/dL, respectively, compared to the healthy control group (M1), which recorded values of 79.48, 76.02, 12.13, and 15.89 mg/dL, respectively.

The findings also revealed that the rat groups treated with bisphenol M2 had significantly lower levels of high-density lipoprotein (HDL), with 29.41 mg/dL, than the healthy control group M1, which had 48.00 mg/dL. When comparing the M2 group to which bisphenol A was added alone, the results also demonstrated that adding nano-chitosan at the stated doses with bisphenol resulted in a drop in the values of triglycerides, cholesterol, LDL, and vLDL and an increase in the HDL value.

Table 3 : Effect of oral administration of CS-NPs on lipid profile (mg/dL) of rats after 28 days

Treatments	TG	TC	HDL	LDL	vLDL
M1	**0.5 ±79.48d**	**0.5 ±76.02d**	**0.05 ±48.00 a**	**0.05 ± 12.13 d**	**0.05 ±15.89 d**
M2	**0.5 ±172.03a**	**0.5±183.21 a**	**0.05 ± 29.41d**	**0.5 ± 119.40 a**	**0.05 ± 34.40 a**
M3	**0.05 ± 112.06b**	**0.6±116.84 b**	**0.05 ± 40.33 b**	**0.05 ± 54.1 b**	**0.05 ± 22.41 b**
M4	**0.05 ± 93.01c**	**0. 6±109.05 c**	**0.5 ± 36.82c**	**0.5 ± 53.63 c**	**0.05 ± 18.60 c**

Different letters in the same column indicate significant differences at a significance level ($p \leq 0.05$) between the treatments.

M1: Control ،M2: Bisphenol-A

M3: CS-NPs 30 % ،M4: CS-NPs 40 %.

Exposure to BPA leads to an imbalance in hormones that regulate or impact the endocrine system, which in turn causes an increase in cholesterol content. Given that research has shown a strong correlation between BPA and metabolic problems, the findings supported the idea that exposure to BPA contributes significantly to the decline of fat metabolism. The significant increase in intracellular fat content is the result of exposure to bisphenol A, which inhibits fat secretion and oxidation. Exposure of experimental animals to BPA can lead to the direct expression of genes involved in fat formation and cholesterol synthesis, thus causing the accumulation of liver fat [18].

These findings are in line with those of [20], who found that administering chitosan nanoparticles to lab animals significantly raised HDL levels while significantly lowering total cholesterol, triglycerides, LDL, and vLDL levels. Triglycerides are transported and accumulated in the liver by vLDL, a barrier. This function might be seen as the initial stage of cardiovascular disease development and incidence.

This explains the lipid-lowering effect of chitosan [21, 22], showed that chitosan treatment considerably reduced the buildup of body fat and dramatically increased the level of lipid and cholesterol in the feces of experimental animals. Chitosan nanoparticles generally demonstrated their ability to lower serum cholesterol levels, enhance lipid metabolism, and shield the hepatic cells' plasma membrane.

CONCLUSION

Chitosan nanoparticles (CS-NPs) at varying concentrations were shown in this study to be able to lessen the toxicity of bisphenol A and its effects on the rates of lipid profiles, liver enzymes, urea, and creatinine in white male rats. In fact, CS-NPs caused these rates to approach their normal levels.

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