

A Hematophysiological and Organotypic Investigation of Albino Rats' Protection Against Bisphenol A-Induced Toxicity Using Chitosan Nanoparticles (CS-NPs)

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Abstract:

The aim of this study was to determine the effect of oral administration of 30% and 40% chitosan nanoparticles on Bisphenol-A (BPA) on liver, spleen and kidney weights, hemoglobin (Hb) concentration, red blood cell count (RBCs), packed cell volume (PCV), platelet count, white blood cell count (WBCs), lymphocyte count, and monocyte count in male albino rats. Bisphenol-A caused a significant decrease ($P \leq 0.05$) in the weights of internal organs (liver, spleen and kidneys), hemoglobin concentration, red blood cell count, packed cell volume (PCV), platelet count, and lymphocyte count in male albino rats. When treated with chitosan nanoparticles, there was a significant decrease ($p \leq 0.05$) in the number of white blood cells and a significant increase ($p \leq 0.05$) in the weight rates of internal organs (liver, spleen, and kidneys), hemoglobin concentration, red blood cell count, packed cell volume (PCV), platelet count, and the percentage of both lymphocytes and monocytes.

Keywords: Chitosan Nanoparticles, Bisphenol A, Red Blood Cell, White Blood Cell.

1. Introduction

Nanoparticles are particles ranging in size from 1-100 nanometers. They are characterized by their physical and chemical properties, such as magnetism, strength, color, solubility, and diffusivity. A variety of techniques can be used to produce nanoparticles, including biological, chemical, and physical techniques. Nanoparticles are highly effective antimicrobial agents that inhibit the growth of various resistant microbes. They are also characterized by their heat resistance and low toxicity (Ali et al., 2025).

Numerous agricultural, medicinal, pharmacological, and environmental applications may benefit from the utilization of chitosan, a naturally occurring biopolymer polysaccharide made by nitrogen deacetylating chitin (Kean et al., 2010). A variety of bacteria are susceptible to the antibacterial properties of chitosan (No et al., 2002). Antitumor activity is one of its many biological qualities (Karagozlu & Se-Kwon, 2014).

Chitosan nanoparticles are characterized by their daily requirement for the body to carry out various metabolic processes. They are an organic particle with the ability to inhibit microbes and have no harmful side effects resulting from their use. Thanks to the biological and mucosal resonance properties of chitosan nanoparticles, they can increase the permeability of the mucous membrane. They are also natural antimicrobial and biological materials, environmentally friendly, with biological activity that is harmless to humans. They are antioxidants and have the ability to eliminate chelated metal ions and free radicals (Ali et al., 2025).

BPA is used in the production of polyvinyl chloride (PVC) plastics, epoxy resins, and polycarbonate. BPA was first synthesized in 1891, but its use did not become widespread until scientists developed polycarbonate plastic using bisphenol A. Paints, dairy packaging, primers, milk bottles, water bottles, and plastic dishes are all made from epoxy resins and polycarbonate plastic. BPA migrates from the packaging material into the food inside the



package (Yang et al., 2018). Mishandling, heating, contact with alkaline or acidic materials, and exposure to microwave radiation can all lead to BPA leaching into food.

Unconjugated BPA is transmitted to the serum of adults and fetuses through food interaction, according to multiple studies. BPA absorption into food is thought to be the most hazardous since many people are exposed to extremely small levels of it and it is difficult to detect it over an extended period of time (Almeida et al., 2018). BPA is classified as an endocrine-disrupting chemical and has been classified as "moderately toxic" by the US Environmental Protection Agency and the European Commission (Flint et al., 2012). A number of studies have confirmed the adverse effects of BPA on growth, reproduction, metabolism, cardiovascular system, neurological function, and immune structures in humans and rodents (Heba et al., 2021).

1.1 Research Problem

The main problem of the research lies in the risks resulting from the accumulation of bisphenol A in food, which is transferred directly from food storage containers to the food itself, posing a threat to human health and causing damage to various internal organs, such as the kidneys, liver, and spleen, in addition to weakening the body's immune system and causing blood diseases.

1.2 Research Objectives

The research aims to use modern, low-cost technologies to reduce the risk of bisphenol A (BPA) by reducing its accumulation in the body's internal organs. Nanotechnology, represented by chitosan nanoparticles, was used to eliminate this hazardous substance from the human body.

2. Related Work

Recent studies have indicated that bisphenol A is an industrial and commercial chemical widely used in the manufacture of beverage containers, epoxy resins, and polyethylene plastics (Lan et al., 2017; Chen et al., 2017). Bisphenol A is frequently detected and has become a serious health problem due to its ubiquitous presence in the environment, food, and drinking water (Djordjevic et al., 2019). Recent research and studies have shown the toxic effects of bisphenol on physical and behavioral factors such as loss of movement, loss of appetite, and the appearance of molecular abnormalities in various tissues such as the liver, brain, kidneys, and reproductive organs of animals (Kumari and Khare, 2018).

3. Methodology

This experiment was conducted in the animal house of the College of Veterinary Medicine and the graduate laboratories of the College of Agriculture, Tikrit University, to determine the effects of chitosan nanoparticles on laboratory animals that were orally administered bisphenol A. Twenty healthy adult male albino rats were used, and the veterinarian confirmed their disease-free status. These rats were purchased from the College of Veterinary Medicine, Tikrit University. They were Sprague-Dawley weanling rats, 9-10 weeks old, and weighed 210-225 grams. They were randomly assigned to four groups, each containing five animals. These animals were:

- 1) Group 1 (M1): The negative control group (healthy) animals were given standard food and drinking water without any additives.
- 2) Group 2 (M2): The infected animals were given 2 ml of Bisphenol A at a concentration of 10 mg/kg animal/day.
- 3) Group 3 (M3): The infected animals were given 2 ml of Bisphenol A at a concentration of 10 mg/kg animal/day + 2 ml of CS-NPs at a rate of 30%.
- 4) Group 4 (M4): The infected animals were given 2 ml of Bisphenol A at a concentration of 10 mg/kg animal/day + 2 ml of CS-NPs at a rate of 40%. The purpose of using two concentrations is to determine which is more effective.

The aforementioned chemicals were administered to laboratory animals after being dissolved in the amounts listed in the aforementioned categories and mixed with sterile water (Rantala, 1974). One day after the experimental animals were fed separately at a temperature of 20–25°C and exposed to lights for at least 12 hours each day, their initial weight was measured. Ad libitum food was served, cooked in accordance with (NAS-NRC, 2002). Male albino rats were given a 20-hour fast and chloroform anesthesia at the conclusion of the experiment, which took place within the allotted 28-day time frame. Following that, blood was extracted straight from the heart (Burtis et al., 2021), to perform the necessary tests, approximately 3-5 ml of blood was drawn into blood collection tubes containing gel and centrifuged at 3000 rpm for 15 minutes to obtain serum, which was kept at the appropriate temperature -20°C until laboratory tests were performed. Laboratory animals were also dissected to obtain their internal organs, including the liver, spleen, and kidneys, and weighed on a sensitive balance.

The data were statistically analyzed using the experimental system within the ready-made statistical program (SAS, (2001) and using the completely randomized design system (CRD), as the means were chosen according to what was stated in Duncan's multi-range test (Duncan, (1955) to determine the significance of the differences between the means of the factors affecting the studied traits at the significance level ($P \geq 0.05$).

4. Result Discussion

4.1 The impact of chitosan nanoparticles (CS-NPs) given orally on the relative weight of a few internal organs in rats

The impact of oral chitosan nanoparticle (CS-NP) treatment on the weights of the liver, spleen, and kidneys of rats given bisphenol A for 28 days is displayed in Table 1. The internal organ weights of the rats treated with bisphenol A for M2 were found to be significantly lower ($P \leq 0.05$) at 5.97, 1.19, and 1.24 g/100 g of body weight, respectively, than those of the healthy control group (M1), which were 9.26, 1.79, and 2.07 g/100 g of body weight, respectively. The results also showed a significant increase in the weights of internal organs when nano-chitosan was added at the above concentrations with bisphenol A compared to the M2 group to which bisphenol A was added alone.

Table 1: Effect of oral administration of CS-NPs on the relative weight of some rat organs, after 28 days of rearing.

Treatments	Liver	Spleen	Kidney
M1	a 0.5 ± 9.26	a 0.06 ± 1.79	a 0.06 ± 2.07
M2	d 0.06 ± 5.97	d 0.05 ± 1.19	cd 0.05 ± 1.24
M3	c 0.5 ± 7.05	c 0.04 ± 1.57	c 0.06 ± 1.89
M4	b 0.45 ± 8.89	ab 0.05 ± 1.69	ab 0.5 ± 2.01

Different letters in the same column indicate to significant differences at ($P \leq 0.05$).

Consumption of bisphenol A by male white rats resulted in a significant decrease ($P \leq 0.05$) in the weight rates of their internal organs due to its toxic effects on the liver, spleen and kidneys as a result of its effect on fat metabolism in these laboratory animals (Chao et al., 2020).

According to (Elshazly et al., 2022), treatment with chitosan nanoparticles significantly increases the weights of the internal organs of male white rats. These results support that finding. The accumulation of nanoparticles in the internal organs of the experimental animals over the experimental period is the reason for the increase in their weights, according to Bhanuramya et al. (2017).

4.2 The effect of oral administration of chitosan nanoparticles (CS-NPs) on red blood cell parameters in rats

Table 2 shows the effect of oral administration of chitosan nanoparticles (CS-NPs) on the red blood cell count of rats treated for 28 days. The results showed a significant decrease ($P \leq 0.05$) in hemoglobin concentration, red blood cell count, packed cell volume (PCV), and platelet count in rats treated with bisphenol A (M2), reaching 9.81 g/dL, $3.62 (\times 10^6/\text{mm}^3)$, 32.08%, and 205 ($\times 10^3/\text{mm}^3$), respectively, compared to the healthy control group (M1), whose parameters were 16.02 g/dL, $6.71 (\times 10^6/\text{mm}^3)$, 45.93%, and 408 ($\times 10^3/\text{mm}^3$), respectively.

While a significant increase in the above parameters was observed when treated with nano chitosan in groups M3 and M4, with hemoglobin levels at 11.74 and 14.67 g/dL, red blood cell count (RBC) at 4.89 and 5.97 ($\times 10^6/\text{mm}^3$), packed cell count (PCV) at 38.91 and 43.15%, and platelet count at 329.6 and 398.7 ($\times 10^3/\text{mm}^3$), respectively, compared to the rats treated with bisphenol A (M2).

Table 2: Effect of oral administration of MgO NPs on red blood cell parameters of rats after 28 days of care.

Treatments	Hb (g/dl)	RBCs($\times 10^6/\text{mm}^3$)	Hem. (PCV) %	Plat. ($10^3/\text{mm}^3$)
M1	a 0.05 $\pm$ 16.02	a 0.05 $\pm$ 6.71	a 0.5 $\pm$ 45.93	a 0.05 $\pm$ 408
M2	d 0.5 $\pm$ 9.81	c 0.04 $\pm$ 3.62	d 0.05 $\pm$ 32.08	d 0.06 $\pm$ 205
M3	c 0.17 $\pm$ 11.74	b 0. 6 $\pm$ 4.89	c 0.5 $\pm$ 38.91	c 0.5 $\pm$ 329.6
M4	b 0.46 $\pm$ 14.67	ab 0.05 $\pm$ 5.97	ab 0.6 $\pm$ 43.15	b 0.06 $\pm$ 398.7

Different letters in the same column indicate to significant differences at ($P \leq 0.05$).

Male albino rats exposed to bisphenol A experienced a marked reduction in red blood cell parameters due to disruptions in the formation of these cells (Sanghamitra et al., 2017), and bisphenol A contributes to mitochondrial dysfunction (Asahi et al., 2010). Hemolysis brought on by reactive oxygen species produced by bisphenol A may be the reason for the notable drop in the blood's hemoglobin percentage and red blood cell parameters (Hager et al., 2020).

These results are consistent with what was stated by (Hamdy et al., 2023) that treatment with chitosan nanoparticles has a noticeable effect in achieving a significant increase in blood hemoglobin parameters, red blood cell count (RBCs), packed cell volum (PCV), and platelet count. Nanoparticles cause an inflammatory response, which means an increase in red blood cell parameters (Hauck et al., 2010).

A decrease in the rate of hemoglobin synthesis or an increase in the rate of hemoglobin oxidation might result in low blood hemoglobin levels. Iron is needed for this process and can be obtained through food and ferritin storage. In experimental animals, iron deficiency is caused by an imbalance or lack of food intake, which lowers the amount of iron available for hemoglobin synthesis (Mohamed et al., 2018). (Cora et al., 2020) verified that bisphenol A exposure in lab animals impairs the neuroendocrine system, which is crucial for controlling food intake and results in metabolic abnormalities. Hemolysis of blood cells brought on by bisphenol A poisoning is the cause of low red blood cell counts and the drop in hemoglobin levels. Speath (2008). (Olson et al., 2000) showed that when examining blood toxicity, higher or lower blood levels signify either an imbalance between the formation and destruction of blood cells or an increase in blood cell levels or their inhibition.

4.3 The effect of oral administration of CS-NPs on the number of white blood cells in rats

The effect of oral administration of CS-NPs on total white blood cell counts is shown in Table 3. The results showed a significant increase ($P \leq 0.05$) in the number of white blood cells in rats treated with bisphenol A (M2), reaching 9.09 ($\times 10^3/\text{mm}^3$) compared to the healthy control group (M1), which reached 6.76 ($\times 10^3/\text{mm}^3$), and a

significant decrease in the percentage of both lymphocytes and monocytes, reaching 24.03% and 5.28%, respectively, compared to the healthy control group (M1), which reached 38.12% and 9.74%, respectively.

While a significant decrease in the above parameters was observed when treated with nanochitosan at different concentrations in groups M3 and M4, the white blood cell counts were 7.81 and 5.97 ($\times 10^3/\text{mm}^3$), respectively. There was a significant increase in lymphocytes, reaching 28.89 and 34.57%, and in the percentage of monocytes, reaching 7.75 and 8.81%, respectively, compared to the rat groups treated with bisphenol A (M2).

Table 3: Effect of oral administration of nanochitosan on the white blood cell counts of rats after 28 days.

Treatments	WBCs($\times 10^3/\text{mm}^3$)	Lymphocytes%	Monocytes%
M1	c 0.04 $\pm$ 6.76	a 0.06 $\pm$ 38.12	a 0.05 $\pm$ 9.74
M2	a 0.06 $\pm$ 9.04	d 0.02 $\pm$ 24.03	d 0.5 $\pm$ 5.28
M3	b 0.05 $\pm$ 7.81	c 0.6 $\pm$ 28.89	c 0.6 $\pm$ 7.75
M4	d 0.05 $\pm$ 5.97	ab 0.06 $\pm$ 34.57	b 0.23 $\pm$ 8.81

Different letters in the same column indicate to significant differences at ($P \leq 0.05$).

The percentage of lymphocytes in laboratory animals exposed to bisphenol A (BPA) significantly decreases because of the higher rate of DNA damage in these cells brought on by an increase in free radical generation linked to oxidative stress. (Rabia et al., 2020). Its capacity to trigger inflammatory conditions, stress brought on by BPA, and boost the immune system can account for the notable rise in white blood cell counts in male albino rats exposed to BPA (Alabi et al., 2021).

Activation of the immune system and defense mechanism against the effects of toxicity can result in an increase in the number of white blood cells as a result of bone tumors or any type of cytotoxicity. Increased white blood cell levels are indicative of an inflammatory response, typically caused by infection. These findings are in line with the findings of (Hamdy et al., 2023), who observed that the application of chitosan nanoparticles resulted in a notable improvement in the proportion of monocytes and lymphocytes and a notable drop in the quantity of white blood cells.

5. Conclusion

The toxicity of bisphenol A and its effects on the relative weight of certain internal organs (liver, spleen, kidney), red blood cell parameters, and white blood cell counts in male albino rats were shown to be lessened by chitosan nanoparticles (CS-NPs) at varying concentrations. These levels were brought nearly back to normal with the usage of CS-NPs.

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دراسة هيماتوفيزيولوجية ونسجية لأعضاء الجرذان البيضاء حول الحماية من السمية المستحثة بمادة بيسفينول A باستخدام جسيمات الكيتوسان النانوية (CS-NPs)

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الملخص:

هدفت هذه الدراسة إلى تحديد تأثير الإغطاء الفموي لجسيمات الكيتوسان النانوية بتركيزي ٣٠٪ و ٤٠٪ على مادة بيسفينول A (BPA) في كل من أوزان الكبد والطحال والكلية، وتركيز الهيموغلوبين (Hb)، وعدد كريات الدم الحمراء (RBCs)، وحجم الخلايا المضغوطة (PCV)، وعدد الصفائح الدموية، وعدد كريات الدم البيضاء (WBCs)، وعدد الخلايا اللمفاوية، وعدد الخلايا الوحيدة في ذكور الجرذان البيضاء. وقد تسبب بيسفينول A في انخفاض معنوي ($P \leq 0.05$) في أوزان الأعضاء الداخلية (الكبد، الطحال، والكلية)، وفي تركيز الهيموغلوبين، وعدد كريات الدم الحمراء، وحجم الخلايا المضغوطة (PCV)، وعدد الصفائح الدموية، ونسبة الخلايا اللمفاوية في ذكور الجرذان البيضاء. وعند المعاملة بجسيمات الكيتوسان النانوية، لوحظ انخفاض معنوي ($P \leq 0.05$) في عدد كريات الدم البيضاء، وارتفاع معنوي ($P \leq 0.05$) في معدلات أوزان الأعضاء الداخلية (الكبد، الطحال، والكلية)، وتركيز الهيموغلوبين، وعدد كريات الدم الحمراء، وحجم الخلايا المضغوطة (PCV)، وعدد الصفائح الدموية، والنسبة المئوية لكل من الخلايا اللمفاوية والخلايا الوحيدة.

الكلمات المفتاحية: جسيمات الكيتوسان النانوية، بيسفينول A، كريات الدم الحمراء، كريات الدم البيضاء.