Uttar Pradesh Journal of Zoology

Volume 44, Issue 24, Page 209-217, 2023; Article no.UPJOZ.3093
ISSN: 0256-971X (P)

The Effect of Oral Doses of Chitosan-Titanium Nanoparticles on Some Physiological Parameters of Male Albino Rats Infected with Acrylamide

Maha Khalil Hamoud [a*], Muhammed Jamil Muhammed [b] and Amin Suleiman Badawi [b]

[a] Department of Food Science, College Agricultural, Iraq.
[b] Department of Food Science, College Agricultural, University of Tikrit, Iraq.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2023/v44i243828

Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Sushma Ishwar Havanur, University of Horticulture Sciences, India.
(2) Bhagyashree Goswami, Hygia Institute of Pharmacy, India.

Original Research Article

Received: 06/10/2023
Accepted: 12/12/2023
Published: 26/12/2023

ABSTRACT

The study aimed to determine the characteristics of nanoparticles, such as shape, size, aggregates, absorption using appropriate equipment, and determine the effect of dosing with chitosan-titanium nanoparticles on some physiological parameters of male rats exposed to acrylamide. We notice a significant decrease in the total number of red blood cells, hemoglobin, the percentage of red blood cells, the number of platelets, lymphocytes, and HDL in the group of rats exposed to acrylamide compared with the healthy control group, as well as a significant increase in the values of white blood cells, monocytes, cholesterol, triglycerides, and LDL. and VLDL compared with the control

**Corresponding author: Email: maha.kh.hmoud@st.tu.edu.iq;*

group. ((While we notice, when adding nanoparticles with acrylamide, a significant increase in the total number of red blood cells, hemoglobin, the percentage of red blood cells, the number of platelets, lymphocytes, and HDL. We also notice a decrease in the numbers of WBC, monocytes, cholesterol, TG, LDL, and VLDL compared to the group exposed to acrylamide.)) While we notice that when nanoparticles are added with acrylamide, there is a significant increase in the total number of red blood cells, hemoglobin, the percentage of red blood cells, the number of platelets, lymphocytes, and HDL, as they were 6.16, 12.00, 45.00, 640.00, 31.00, 37.15, respectively, compared to the group. exposed to acrylamide, which were 4.73, 9.65, 38.50, 588.50, 25.60, 32.65, respectively. We also note a decrease in the numbers of WBC, monocytes, cholesterol, TG, LDL, and VLDL, as they were 6.10, 7.90, 158.75, 99.75, 101.65, and 19.95 compared to those exposed to acrylamide. The group exposed to acrylamide, which was 7.67, 14.95, 197.60, 171.29, 130.68, 34.26, respectively.

Keywords: Chitosan-titanium nanoparticles; acrylamide; physiological and histological parameters.

1. INTRODUCTION

Chitosan nanoparticles can be defined as natural materials that have good physicochemical properties, are antibacterial, have biological activity, and are suitable for human use, making them an environmentally friendly material [1]. The distinctive and unique properties of chitosan nanoparticles have led to many applications in different fields. Chitosan has the activity of removing free radicals and masking metal ions, due to the small size and low molecular weight of the nanoparticles, which contribute to giving the ability to nano-chitosan as an antioxidant (Divya *et al.,* 2017). The surface area and surface-to-volume ratio increase, which in turn leads to increased solubility, which increases the active effect of chitosan in the body (bioavailability) [2].

Chitosan is soluble in dilute mineral and organic acids, but precipitates at a pH above 6.0. It acts as an ion exchange resin and must bind hydrophobic ions, such as bile acids, at a low pH level similar to that found in the intestine [3]. Chitosan nanoparticles, due to their biological and mucoadhesive properties, can improve mucosal permeability, thereby enhancing transport through the cellular pathway of nanoparticles, and can induce structural reorganization of proteins [4].

Titanium is a non-toxic material used in human food, medicines, and food contact materials. It is used as bleaching agents in various applications such as paints, cosmetics, and food products. It is one of the most widely used inorganic metal oxides in environmental applications to remove various pollutants from water and air [5]. Zang et al. [6] incorporated titanium dioxide nanoparticles into chitosan membranes and used it to package red grapes and store them for six days. The results showed exceptional performance in protecting grapes from microbial attack and prolonging the shelf life of red grapes, in addition to eliminating microorganisms within 12 hours of exposure time.

Acrylamide (H2C=CH-NH2) is one of the compounds that are formed in foods rich in starchy substances and that are exposed to high heat treatments during manufacturing [7]. It is a white, crystalline, odorless substance that is formed in baked and fried foods as a result of exposure to high heat treatments. It has been found that It has a negative impact on human health, as it is formed by heating at a temperature above 120°C as a result of Maillard reactions between the amino acid asparagine with glucose or any other sugar. Acrylamide is also found in high concentrations in many foodstuffs, primarily French fries and potato chips, followed by cereals, brittle bread, and products. other baked goods [8]. Acrylamide is a white, crystalline, odorless substance that is formed in baked and fried foods as a result of exposure to high heat treatments. It has been found to have a negative effect on human health, as it is formed by heating at a temperature above 120°C as a result of Maillard reactions between the amino acid asparagine with glucose or any other sugar [8]. (ACR) is a chemical formed in cooked starchy foods such as potatoes and bread. High levels of acrylamide have carcinogenic, genotoxic, neurotoxic and hepatotoxic effects on living organisms.

2. METHODOLOGY

Production of chitosan-titanium nanoparticles: Chitosan-titanium nanoparticles were prepared by adding 10 mg of titanium nanoparticles (TioNPs) previously produced by a biosynthesis method using the *Aspergillus niger* fungus to 1 g of chitosan powder and adding 10 ml of distilled water in an aluminum-coated glass beaker while continuing to stir using a magnetic stirrer at a temperature Low for 24 hours until the color change is obtained, which is an initial indicator of the formation of nanoparticles.

Preparing and examining nanoparticle samples:

Preparing the sample for examination of the absorption spectrum of visible and ultraviolet rays Absorption UV-Visible Light Spectroscopy: The sample was prepared for examination by placing the nano solution in the incubator for 72 hours at a temperature of 26-25 °C and in a dark envelope, after which 2 ml of the nano solution was taken and shaken well to homogenize the solution and examined with a Visible UV Spectroscopy device [9].

Prepare the sample for examination with a scanning electron microscope:

Scanning Electron Microscopy (SEM): The sample was prepared for examination under the microscope using a copper mesh covered with carbon. A very small amount of the sample was placed on this mesh and examined with a scanning electron microscope to determine the morphological composition of the produced particles [10].

Preparation of laboratory animals: Adult male Albino rats were obtained from the College of Veterinary Medicine/University of Tikrit at 7-8 weeks of age. Their weights ranged between 125-160 grams. They were randomly distributed into three groups with similar weights, with three animals in each group. The rats were fed for 5 days on the basic diet on a regular basis using a prepared diet according to [11]. The essential groups and solutions of acrylamide and nanoparticles were prepared as shown below:

- Control group Without treatment (T1).
- The acrylamide treated group: the solution Prepared at a concentration of 40 mg/kg/day. : (T2)
- The group treated with acrylamide + chitosan-titanium nanoparticles (T3):the solution Prepared at a concentration of 500 mg/kg/day.

Blood analysis: After the end of the experiment, the animals were starved for 10 hours after the end of the experiment and anesthetized using chloroform, then the rats were dissected from the chest area and blood was drawn from the heart into two tubes, the first containing the anticoagulant EDTA to measure blood picture, and the second devoid of it, containing 2-3 ml of blood, which was centrifuged by using a Centrifuge device at a speed of 3000 rpm for 15 minutes, then the serum was taken and stored at a temperature of -20 °C until the necessary analyzes were performed, according to [12].

Then the infected animals in group T3 were treated with chitosan-titanium nanoparticles at a concentration of 500 mg/kg body weight for 28 days. After completing the experiment, the body weights of the male rats used before the experiment were measured to calculate the initial weight, and the difference in weight was extracted as in the following equation:

Weight gained (g) = final weight (g) - starting weight (g).

Statistical analysis; The data were statistically analyzed using the complete random design (CRD) system in the experimental system within the ready-made statistical program (SAS, 2012), and the averages were chosen according to Duncan's multiple range test [13] to determine the significance of the differences between the averages of the factors affecting the studied characteristics at the level of (0.05).

3. RESULTS AND DISCUSSION

Absorption UV-Visible light spectroscopy: The absorption peak Chitosan - titanium nanoparticles (Ch-Tio NPs) was at a wavelength of 200 nm for chitosan. The result was close to what was found by Agarwal et al., [14] that the wavelength of chitosan particles ranged between 201 - 216 nm, and it was 202 nm for titanium nanoparticles when combined with chitosan particles. This indicates the high ability of titanium nanoparticles to bind with chitosan particles and form a complex of chitosan and titanium nanoparticles due to the electronically excited titanium particles and because chitosan contains primary and secondary hydroxyl groups at the positions of the C-2 and C carbon atoms. - 6 and 3C, respectively, which enables it to form hydrophobic hydrogen and ionic bonds with other molecules. Also, the titanium nanoparticles are positively charged and their electrons are excited, so they combine with the negatively charged hydroxyl groups of chitosan and form nanoparticles.

Scanning Electron Microscopy (SEM): The results obtained from the scanning electron microscope (SEM) as shown in Fig. (1) (A, B, C, and D) and obtained at different magnification powers showed the distribution of chitosan-titanium nanoparticles in the form of granules with a size range ranging between 33.74 - 34.34 nanometers, where these particles were homogeneous. Distribution in non-contiguous clusters. As shown in the micrograph of the shapes (D), they were in the form of spherical granules.

Effect of chitosan-titanium nanoparticles on the weight of acrylamide-infected rats: Table (1) shows the effect of oral administration of chitosan-titanium nanoparticles to animals on the weight of rats exposed to acrylamide for 28 days. The results showed a significant decrease in weight ($P \leq 0.05$) in the weight gained for the groups of rats treated with acrylamide T2 compared to the untreated control group T1, which was 16.28-27.97 grams, respectively. While a significant increase in weight was observed at ($P \leq 0.05$) in the group to which Ch-TiO2 (T3) was added, which was 11.91 g compared to the group of rats treated with acrylamide T2.

The results agreed with the findings of Wang et al. [15] that acrylamide has a bad effect on the weight and growth of male rats because it has harmful effects that cause a deficiency of body protein. Abdul-Hamid *et al.,* (2007) indicated that low body weight is due to excessive breakdown of proteins. The low weight of animals fed acrylamide compounds may be linked to the toxicity of these compounds, as they affect their growth, and the effect of this toxic substance on the effectiveness of digestive enzymes in the intestines and lead to an imbalance in metabolic processes, which in turn affects the endocrine glands and causes an imbalance in the overall body weight. [16].

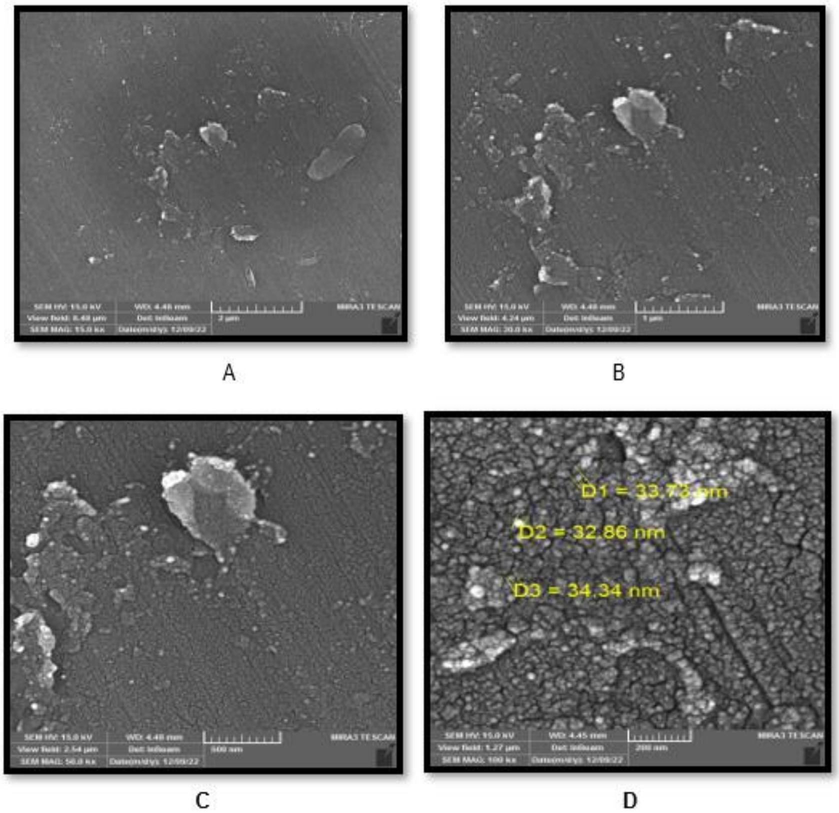

Fig. 1. SEM images of chitosan nanoparticles, Ch-TiO2-NPs, prepared from the filtrate of the fungus A. niger, with different dimensions

A: Ch-NPs with an magnification of KX 15.0 and a size WD: 8.48 mm, B: Ch-NPs with an magnification of KX 30 and a size WD: 4.24 mm, C: Ch-NPs with an magnification of 50.0 KX and a size WD: 2.54 µm, D: Ch particles -NPs with magnification power of 100KX and size of 1.27 WD: µm.

Table 1. Effect of chitosan-titanium nanoparticles on the weight of animals exposed to acrylamide after 28 days

Features / Groups	Average animal weights (g)		
	Primary	**Finally**	**Gained weight**
T1	0.89 ± 142.47	1.83 ± 170.45	0.93 ± 27.97a
T2	2.13 ± 147.79	2.06 ± 131.50	0.06 ± 16.28 -c
T3	2.93 ± 143.19	2.47 ±162.11	0.46 ± 18.19b

Increased secretion of thyroid hormone leads to wasted energy and weight loss, while decreased secretion of thyroid hormone leads to increased energy and increased fat, thus increasing body weight. While we notice that when rats infected with acrylamide take chitosan-titanium nanoparticles at a concentration of 500 mg/kg of body weight, their weight increases and they return to their normal state gradually, because chitosan is an antioxidant agent [17], so it can get rid of free radicals and metal ions by donating hydrogen or One pair of electrons (Lin *et al.,* 2009).

The amino and hydroxyl functional groups of chitosan react with metal ions leading to a number of activities such as adsorption and ion exchange (Onsosyen and Skaugrud, 1999). Yen *et al.,* [18] reported that chitosan showed strong hydroxyl radical activity and its ability to chelate iron.

Impact on blood picture: Table (2) shows the effect of oral administration of Ch-TiO2 NPs on the blood image parameters of male rats exposed to acrylamide for 14 days. It was found that there was a decrease in the total number of red blood cells, hemoglobin, and the percentage of red blood cells and platelets in the group treated with acrylamide, as their values were (4.73, 9.65, 38.50, and 588.50), respectively, compared with the control group, whose values were (6.66, 13.40, and 51.50). and 892.00) respectively.

While there was an increase in the total number of red blood cells, hemoglobin, and the percentage of red blood cells and platelets in the group treated with Ch-TiO2 NPs, as their values were (6.16, 12, 45, and 640), respectively, compared to the group treated with acrylamide alone.

Exposure the body to acrylamide leads to the occurrence of anemia due to a decrease in the number of red blood cells and hemoglobin, as it leads to a shortened life span due to an increase in the rate of mechanical crushing of the cell membranes, with inhibition of the sodium and potassium ATP enzymes, which have an important role in the formation of red blood cells, and also affect It affects the metabolism of iron through its action in inhibiting the enzyme ALA dehydrogenase, which participates in the last step of iron formation and is considered the basic component of hemoglobin [19].

The reason for the low level of hemoglobin concentration in acrylamide-fed treatments is due to its association with albumins responsible for manufacturing (Hb) in the blood and responsible for the synthesis of red blood cells, and thus affecting the ability to absorb iron in the intestine, which is considered one of the main components of hemoglobin [20].

The increase in red blood cells in rats which given Ch-TiO2 NPs is due to its containing active groups such as the positive charge that is due to the presence of free amino groups attached to the second carbon atom of the glucosamine sugar, and it also contains two primary and secondary hydroxyl groups in the carbon atom. C-2, C-6, and C-3, which makes it easily soluble, as it forms hydrophobic and hydrophobic hydrogen and ionic bonds with other molecules, so it contributes to it being a chelating agent.

Chitosan has biological and chemical properties that create it have many applications in various industrial and medical fields, as it acts to heal bones and wounds, regulate blood cholesterol, skin burns, contact lenses, surgical sutures, an agent in blood clotting and reducing tumors [21]. Ali and Ahmed [22] pointed out the use of chitosan as an anti-bacterial, anti-oxidant, anti-inflammatory and improving blood clotting properties, as a study showed an improvement in blood clotting and enhancement of the rates of other cell characteristics using chitosan/kaolin nanoparticles (NPs), compared to traditional dressings (Elsabahy and Hamad, 2021).

Table 2. Effect of oral administration of Ch-TiO2 NPs on erythrocyte parameters of rats exposed to acrylamide after 28 days (means ± standard error)

Features / Groups	The value of blood picture			
	Hb (g/dl)	RBCs ($\times 10^6/mm^3$)	Hematocrit (PCV) %	Platelets ($10^3/mm^3$)
T1	0.20 ± 12.40a	0.04 ± 6.66a	1.50 ± 51.50a	4.00 ± 892.00a
T2	0.05 ± 9.65c	0.05 ± 4.73c	0.50 ± 38.50c	7.50 ± 588.50c
T3	0.20 ± 12.00b	0.05 ± 6.16b	1.00 ± 45.00b	2.00 ± 640.00d

Table 3 shows the effect of chitosan-titanium nanoparticles on male rats infected with acrylamide on the total number of white blood cells, monocytes, and lymphocytes of male rats. The results showed a significant increase in the number of white blood cells and monocytes (7.67, 14.95) compared to the control group (5.33, 6.85), respectively, when acrylamide was added alone. There was also a decrease in lymphocytes, which was 25.60% compared to the control group, which was 38.55%.

While there was a significant decrease in the number of white blood cells and monocytes and an increase in lymphocytes in the group fed with chitosan-titanium nanoparticles, reaching 6.10, 7.90, and 31 compared to the group treated with acrylamide without treatment. The results agreed with what was reported by Firouzabadi et al. [20]. Which showed that exposure to acrylamide led to a significant increase in the number of white blood cells compared to the healthy control group, and the increase in the number of white blood cells is due to the body's defensive function against foreign bodies that attack it and the work to remove them, as white blood cells are considered the body's first line of defense when exposed to external influences.

The results agreed with what was reported by Chen et al. [23] that oral administration of dietary nano-TiO2 at a concentration of 2.5 mg/kg body weight/day for 7 days led to positive changes in white blood cell parameters, red blood cells, and other parameters in laboratory mice.

Effect of chitosan-titanium nanoparticles on lipid profile parameters of male rats infected with acrylamide: Table (4) shows the effect of oral administration of Ch-TiO2 NPs to male rats for 28 days on lipid profile parameters. We notice a high concentration of cholesterol in the group of rats infected with acrylamide (T2), which reached 197 mg/dL compared to the healthy control group (T1), which amounted to 135 mg/dL, while its concentration decreased in the group treated with Ch-TiO2 (T3) NPs particles, reaching 158. 75 mg/dL 99.75 mg/dL. The concentration of triglycerides (TG) also increased in the infected animal group (T2), reaching 171.29 mg/dL compared to its value in the healthy animal group (T1), which amounted to 81.09 mg/dL, while its values decreased in the T3 group, reaching 99.97 mg/dL compared to the infected group (T2).

We notice a significant decrease in high-density lipoproteins (HDL) in the infected group (T2), reaching 32.65 mg/dL compared to their value in animals of the healthy group (T1), which amounted to 54.35 mg/dL, while their values increased in the group of animals treated with Ch-TiO2NPs. (T3) was 37.15 mg/dL.

While the concentration of low-density lipoproteins (LDL) decreased in the T3 group of treated animals, as it was 101.65 mg/dL, compared to its concentration in the blood of the infected animal group (T2), which was 130.68 mg/dL, which was higher than the concentration of LDL in the healthy control group. (T1) which it was reached to 65.12 mg/dL.

Table 3. Effect of oral administration of Ch-TiO2NPs on the numbers of white blood cells in rats exposed to acrylamide after 28 days

Features / Groups	The value of blood picture		
	WBCs (×103/mm3)	lymphocytes %	WBCs (×103/mm3)
T1	0.02 ± 5.33c	1.05 ± 38.55a	0.02 ± 5.33c
T2	0.03 ± 7.67a	0.80 ± 25.60c	0.03 ± 7.67a
T3	0.12 ± 6.10b	1.40 ± 31.00b	0.12 ± 6.10b

Table 4. Effect of chitosan-titanium nanoparticles on the lipid profile of rats after 28 days

Features / Groups	The value of blood picture				
	TG	**TC**	**HDL**	**LDL**	**vLDL**
T1	1.07 ± 81.09d	2.99 ± 135.69d	0.75 ± 54.35a	2.02 ± 65.12f	0.21 ± 16.21d
T2	6.60 ± 171.29a	0.30 ± 197.60a	1.40 ± 32.65d	0.21 ± 130.68a	1.32 ± 34.26a
T3	0.95 ± 99.75bc	2.55 ± 158.75c	0.95 ± 37.15c	1.79 ± 101.65bcd	0.19 ± 19.95bc

Also, the concentrations of very low-density lipoproteins (VLDL) increased significantly in the group of infected animals (T2), which was 34.26 mg/dL, compared to their concentration in the healthy control group (T1) and were at 16.21 mg/dL, while their concentrations decreased in the group of animals treated with Ch- TiO2NPs (T3) was 19.35 mg/dL compared to its concentration in the T2 group of animals.

One of the causes of high cholesterol in the body is the increased activity of the enzyme cholesterol acyl transferase, which is responsible for the absorption of cholesterol, which is stimulated when there is a lack of insulin due to oxidative stress that affects pancreatic beta cells, and thus the level of cholesterol absorption by the intestine increases (Abdullah, 2010). The increase in cholesterol, triglycerides, and LDL upon oral administration of acrylamide leads to impaired liver function [24], and the increase in cholesterol concentration is also due to a decrease in the activity of the hydroxysteroid dehydrogenase enzyme and a change in the process of steroid synthesis.It also leads to a rapid increase in the concentration of cholesterol and triglycerides in blood serum by inhibiting the enzyme non-specific esterase, pyrophosphatase, and triglyceride lipase, leading to a change in fat metabolism [25].

Treatment with nanoparticles has reduced the concentrations of TC, TG, VLDL, and LDL in rats while increasing HDL concentrations. The reason for the improvement in standards may be due to the content of these substances on active groups that reduce fat accumulation, as well as stimulate the activity of lipoprotein lipase, which leads to a decrease in In TG and may increase the uptake of triglycerides from plasma by skeletal muscle and adipose tissue (Fogelman *et al.,* 2016: Fulianga *et al.,* 2005).

Cholesterol-lowering properties have been attributed to chitosan, especially in rats fed a high-cholesterol diet (Vahouny *et al.,* [26], as consuming chitosan at 5% of the diet reduces blood cholesterol levels to the level of rats fed a normal cholesterol diet (Fukada *et al.,* [27], reducing cholesterol in the blood of experimental animals is achieved by binding cholesterol to the presence of bile acids and forming the cholesteramine compound, and cholesterol is eliminated from the body through feces (Grundy *et al.,* 1971), previous study confirmed that chitosan has low toxicity, It is safe in mice at concentrations up to 10% in the diet [28].Some studies indicate that cholestyramine is associated with colon cancer in humans [29] and rats [30].

It has been assumed that reducing cholesterol with chitosan occurs through mixing glucosamine with bile acid and cholesterol in the intestinal lumen and thus enters the absorption process [26].

4. CONCLUSION

The chitosan-titanium nanoparticles had a wavelength of 200 nm and were characterized by the shape of spherical particles with sizes ranging from 33.74 - 34.34 nm.Ch-TiO2NPs particles played an important role in getting rid of the body of harmful fats that were formed due to oxidation with the toxic substance acrylamide, and returning the body to its normal state, as well as an increase in the weights of animals treated with Ch-TiO2NPs after their decrease in the affected group, and an increase in the numbers of red blood cells, hemoglobin percentage, and... The percentage of red blood cells and platelets and a decrease in white blood cells and monocytes with an increase in the percentage of lymphocytes in male white rats infected with acrylamide and treated with Ch-TiO2 NPs compared to the group of rats infected with acrylamide without treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kouachi HJ, Jahanian MAG, Berenjian A. Potential applications of chitosan nanoparticles as novel support in enzyme

immobilization. Am J Biochem Biotechnol. 2012;8(4):203-19.
2. Ghadi A, Mahjoub S, Tabandeh F, Talebnia F. Synthesis and optimization of chitosan nanoparticles: potential applications in nanomedicine and biomedical engineering. Caspian J Intern Med. 2014;5(3):156-61.
3. Nagyvary JJ, Falk JD, Hill ML, Schmidt ML, Wilkins AK, and Bradbury EL. Hypolipidemic activity of chitosan and other polysaccharides in rats.1 N&r Rep Int. 1979;20:677-684.
4. Peppas NA, Huang Y. Nanoscale technology of mucoadhesive interactions. Adv Drug Deliv Rev. 2004;56(11):1675-87.
5. Yadav HM, Kim JS, Pawar SH. Developments in photocatalytic antibacterial activity of Nano TiO2: A review. Korean J Chem Eng. 2016;33(7):1989-98.
6. Zang Z, Zeng X, Wang M, Hu W, Liu C, Tang X. Tunable photoluminescence of water-soluble AgInZnS–graphene oxide (GO) nanocomposites and their application in-vivo bioimaging. Sens Actuators B. 2017;252:1179-86.
7. Maan AA, Anjum MA, Khan MKI, Nazir A, Saeed F, Afzaal M et al. Acrylamide formation and different mitigation strategies during food processing–a review. Food Rev Int. 2020:1-18.
8. Konings EJ, Baars AJ, van Klaveren JD, Spanjer MC, Rensen PM, Hiemstra M, Van Kooij JA, Peters PW. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. Food and Chemical Toxicology. 2003 Nov 1;41(11):1569-79.
9. Jayaseelan C, Rahuman AA, Kirthi AV, Marimuthu S, Santhoshkumar T, Bagavan A, Gaurav K, Karthik L, Rao KB. Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012 May 1;90:78-84.
10. Castro-Longoria E, Vilchis-Nestor AR, Avalos-Borja M. Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus Neurospora crassa. Colloids and surfaces B: Biointerfaces. 2011 Mar 1;83(1):42-8.
11. NRC (National Research Council). Nutrient Requirements of Laboratory Animals 4th ed. National Academy Press, Washington, DC; 1995.
12. Tietz Y. Clinical Biochemistry; 6th ed., McGraw-Hill, New York. 2005;825.
13. Duncan DB. Multiple range and F. test. Biometric. 1955;11:42.
14. Agarwal UP, Ralph SA, Reiner RS, Hunt CG, Baez C, Ibach R et al. Production of high lignin-containing and lignin-free cellulose nanocrystals from wood. Cellulose. 2018;25(10):5791-805.
15. Wang H, Huang P, Lie T, Li J, Hutz RJ, Li K et al. Reproductive toxicity of acrylamide-treated male rats. Reprod Toxicol. 2010;29(2):225-30.
16. Chen S, Qiu Z, Lin W. The immunogenicity of ultrasonicated vaccine of chicken pathogenic E. coli (078) against colibacillosis. J S China Agric Univ. 1999;20(1):37-40.
17. Rajalakshmi A, Krithiga N, Jayachitra A. Antioxidant activity of the chitosan extracted from shrimp exoskeleton. Middle East J Sci Res. 2013;16(10):1446-51.
18. Yen MT. yang j .h .and Mau J.L . Antioxid Prop Chitosan Carb Shells Carbohydr Polym. 2008;74:840-4.
19. Benzianea AB, Abdelkader DB, Abdelkader M, Ahmed B, Mohammed B. Effect of oral exposure to acrylamide on biochemical and hematologic parameters in Wistar rats. Drug Chem Toxicol. 2018;9:1-10.
20. Firouzabadi AM, Imani M, Zakizadeh F, Ghaderi N, Zare F, Yadegari M et al. Evaluating effect of acrylamide and ascorbic acid on oxidative stress and apoptosis in ovarian tissue of Wistar rat. Toxicol Rep. 2022;9:1580-5.
21. Akila RM. Fermentative production of fungal chitosan, a versatile biopolymer (perspectives and its applications). Adv Appl Sci Res. 2014;5(4):157-70.
22. Ali A, Ahmed S. A review on chitosan and its nanocomposites in drug delivery. Int J Biol Macromol. 2018;109:273-86.
23. Chen H, Zhao R, Wang B, Cai C, Zheng L, Wang H et al. The effects of orally administered Ag, TiO2 and SiO2 nanoparticles on gut microbiota composition and colitis induction in mice. NanoImpact. 2017;8:80-8.
24. Khalil FA, Abd El-Aziem MA. Effect of dietary acrylamide formed in potato crisps and toasted bread on rats. Egypt. J Nat Toxins. 2005;2:57-70.

25. Benzianea AB, Abdelkader DB, Abdelkader M, Ahmed B, Mohammed B. Effect of oral exposure to acrylamide on biochemical and hematologic parameters in Wistar rats. Drug Chem Toxicol. 2018;9:1-10.
26. Vahouny GV, Satchithanandam S, Cassidy MM, Lightfoot FB, Furda I. Comparative effects of chitosan and cholestyramine on lymphatic absorption of lipids in the rat. Am J Clin Nutr. 1983;38(2):278-84.
27. Fukada Y, Kimura K, Ayaki Y. Effect of chitosan feeding on intestinal bile acid metabolism in rats. Lipids. 1991;26(5):395-9.
28. Furda I. Amino polysaccharides: their potential as dietary fiber. In:Furda 1 (ed) ACS Symposium Series: Unconventional Sources of Dietary Fiber. American Chemical Society, Washington DC, pp 105- The Endocrine Society18. 122, 1983.
29. Hill MJ, Morson BC, Thompson MH. The role of faecal bile acids in large bowel carcinogenesis. Br J Cancer. 1983;48:143.
30. Nigro ND, Bhadrachari N, Chomchai C. A rat model for studying colonic cancer; Effect of cholestyramine on induced tumors. Dis Colon Rectum. 1973; 16(6):438-43.

© Copyright MB International Media and Publishing House. All rights reserved.