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RESEARCH PAPER

The Effect of Copper Oxide Nanoparticles on Hepatic and Renal Toxicity in Domestic Rabbits

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ABSTRACT

The production and use of engineered nanomaterials in various industries, especially the food industry, and its influence in food products have caused concerns in the field of health. The aim of this study is to investigate the toxicity level and evaluate the effects of copper oxide nanoparticles (CuO NPs) on internal tissues. For this purpose, the effect of different doses of copper oxide nanoparticles in the kidney tissue of rabbits was evaluated. In this experimental study, 48 domestic rabbits were randomly divided into 4 groups of 12 cases. One group was considered as the control group, and three groups of rabbits were injected with doses of 5, 15, and 25 mg of oxide nanoparticles in 3 times a day for 15 days. Then, the biochemical factors (Blood Urea Nitrogen), BUN, Uric Acid (UA) and Creatinine (Cr) were measured in the kidney. The kidney tissue was examined in three stages of blood collection (one day, 8 days and 15 days after the treatment). Also, after 15 days, the animals were isolated and the kidney tissue sections were prepared and studied with hematoxylin-eosin staining. The activity of kidney biochemical factors did not show any significant change between the different groups in the blood collection stages, so that in the first blood collection, there was a significant increase in all three treatment groups compared to the control group. The control showed the greatest changes after 15 days (the third blood draw). There was no significant statistical difference in the weight of the rabbits between the different groups. Histological studies showed multiple tissue damage in the treatment groups, including hyperemia, degeneration of hepatocytes, hyperplasia and inflammation. Microscopic results also showed that the most changes in hepatocyte cells, central lobular vein and sinusoidal space and cause tissue damages.

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INTRODUCTION

Nanotechnology is a multidisciplinary scientific branch that includes many sciences, including: biomedical, pharmaceutical, agriculture, environmental science, advanced material science, chemistry, physics, electronics, information technology, etc. [1] to produce nanoparticles from metals such as Gold, silver, iron, platinum, palladium, as well as inorganic non-metallic particles such as zinc oxide and titanium oxide are used because these materials have electrical, mechanical, optical, chemical and magnetic properties [2]. The most important property of nanoparticles is a very high surface to volume ratio. it is them Nanoparticles are used in light electrons, display devices, catalysts, fabric biosensors, identification or monitoring of diseases such as cancer, drug production, removal of metal toxicity or anti-environmental agents, and also in medical services [3-6].

In biomedical, Nano materials are of special importance due to their compatibility with cells (10-100 microns), viruses (20-450 nanometers), proteins (5-50 nanometers) and genes with a width of 20 nanometers and a length (10-100 nanometers) of a sufficiently small size are able to move inside the body without disrupting the body's natural functions and can also reach remote areas [7, 8]. In recent years, many efforts have been made to produce nanoparticles due to their optical, chemical, electrical, and photoelectric properties. It is produced by a simple chemical method, but there is a possibility that toxic substances from the production will remain on the produced nanoparticles. In the physical production method, the produced nanoparticles have low toxicity. But this method is time-consuming and the produced products do not have the same size [9]. Biological and non-toxic production of metal nanoparticles by living microorganisms such as bacteria, fungi and plants has made extensive use of nanoparticles in the industry because they create less toxicity and require less energy to produce them. It is consumed and therefore the cost of its production is also reduced [10]. Also, it is easy to maintain microbes, their growth takes place in inexpensive environments such as cellulose waste, their security level is controlled, they have the potential to absorb metal ions and reduce them to nanoparticles by producing metabolic enzymes [11, 12]. Therefore, biological methods such as the use of microbes, bacteria, fungi and algae, viruses and plants are more suitable. Among these, algae and fungi are mentioned as factories for the production of natural nanoparticles. These biological nanoparticles have a positive effect on the environment, their use is cost-effective, and their unique structure has given them a high metal absorption capability [13, 14]. They are neutralized in microbes and plants. For example, in the case of fungi, nitrate reductase is used in the decreasing of metal ions and the production of nanoparticles [15]. The biochemical properties of nanoparticles, such as size, contact surface, energy level, and absorption are important in binding with biological molecules, and the fate of nanoparticles in determine the cells [16].

In general, due to the many applications of nanomaterials in various industries and the existence of many hypotheses on the harmful effects of nanoparticles on living organisms, investigation and research in this field is of great importance. Due to the fact that all types of nanoparticles may enter the body of living organisms simultaneously or in combination and that each nanoparticle has been studied individually [17], therefore, in the study of the effects of copper oxide nanoparticles on biochemical factors and kidney tissues Rabbits have been analyzed.

MATERIALS AND METHODS

In this experimental and laboratory study, 48 domestic rabbits with a weight of 3-5 kg and a height of up to 60 cm were used. The tested animals were kept under controlled conditions (temperature 53±5, humidity 22±2%, light 05 hours day and 05 hours night). Animals received food and water ad libitum throughout the experiment. In all stages of the research, the ethical principles of working with laboratory animals approved by the animal ethics

Table 1. Characteristics of copper nanoparticles

Feature	Amount
Size	10-20%
Anatase	79.1%
Rutile	21.5%
Specific surface area	100-150 m^2g
Density	3.88 gr/cc

committee of the university were observed.

Copper nanoparticle: 350 ml, copper nanoparticle was purchased from Kazakhstan Nanomaterials Company, which commercially supplies this nanoparticle from France. To ensure the correctness of the dimensions of the nanoparticles, one gram of the nanoparticle was sent with the aforementioned birth certificate, and the correctness of this nanoparticle and its diameter were confirmed by X-ray tests. As mentioned in the previous parts, the copper nanoparticle used in this research has a diameter of 10-20 nm and is spherical in shape with mineral nature and wet synthesis method in the liquid phase (co-precipitation) in solution and had the following characteristics (Table 1.).

The data for four groups of the study collected. These categories include: 1st group) the control group who received 0.5 ml of physiological serum intraperitoneally to equalize the effect of the shock resulting from the injection in the treatment and control groups. The second group was injected with 5 ml of nanoparticles, the third group with 15 ml of copper nanoparticles, and the fourth group with 25 ml of copper nanoparticles intraperitoneally. These injections were done for 15 consecutive days. From the mentioned mice, blood sampling was performed on the first, eighth and fifteenth days after the treatment. Blood was collected from the corners of the eyelids of the animals using a capillary tube. The samples were centrifuged for 5 minutes at 3000 RPM/Minute and their serum was separated. Blood biochemical factors BUN, UA) and Cr were measured using biochemical kits and an Auto-analyzer.

Preparation of kidney tissue sections from control and treated rabbits for staining: After 15 days, a number of control and treated mice from each group were dissected after anesthesia with ether, and tissue sections were prepared from their kidneys for staining with hematoxylin-eosin. SPSS software was used for statistical measurement of the data and all the obtained results were calculated as Mean ± SD.

RESULTS AND DISCUSSION

In the histopathological examination following the use of different doses of nanoparticles on lesions like hyperemia, necrosis, hyaline casts, edematous cells infiltration, glomerular proliferation and fibrosis, it was seen that the most lesions happened in the 25 mg dose group [19, 20]. Due to the negative effects of zinc oxide nanoparticles in proportion to the increase in the dose due to inflammation, hyperemia and fibrosis that occurs in high doses, as a result the tissue of the kidneys is also changed. The measurement of the biochemical values of blood urea nitrogen in the 25 mg group showed a significant difference with the other groups ((P<0.05) Table. 2). Also, in the measurement of the biochemical values of creatinine, the 25 mg group showed a significant difference with the 15 mg group, showed (P<0.05), while no significant difference was seen among other groups (Table 2). Also, measuring the biochemical values of urea, the 588 mg/kg group indicated a significant difference with the other groups.

In the next step, the effect of nano copper oxide accumulation on tissues was evaluated. As seen

Table 2. Comparison of Creatine-urea and uric acid levels in different groups

	Day 1	Day 8	Day 15	P-value
		Ce		
Control	0.63±0.10	0.64±0.10	0.65±0.10	0.001
Group 1	0.65±0.19	0.66±0.13	0.69±0.15	0.000
Group 2	0.67±0.15	0.68±0.11	0.70±0.16	0.002
Group 3	0.71±0.16	0.72±0.18	0.74±0.12	0.004
		UA		
Control	24.11±2.1	25.31±2.56	24.89±3.11	0.000
Group 1	25.19±.9	26.5±2.21	27.68±3.02	0.001
Group 2	26.01±1.4	27.45±1.93	30.07±2.78	0.001
Group 3	27.52±1.9	28.01±2.71	32.65±2.65	0.003
		BUN		
Control	4.31±0.12	4.27±0.18	4.29±0.17	0.000
Group 1	4.27±0.14	4.44±0.13	4.62±0.14	0.001
Group 2	4.31±0.13	4.51±0.11	4.71±0.11	0.001
Group 3	4.35±0.09	4.61±0.16	4.90±0.21	0.003

in Fig. 1, the effect of copper nanoparticle with a concentration of 25 on this tissue is intense. The complete destruction of tubules in the cortex - the destruction of renal corpuscles - the decrease in acidophilic activity in the cells of the walls of the proximal tubules indicates the strong effect of the nanoparticle.

The cells in the body tissues of organisms have different enzymes that work in connection with the specific function of the cell and all the biochemical reactions inside the cells are catalyzed by them. When a cell is damaged, enzymes enter the interstitial fluid. Changes in enzyme activity in rabbits exposed to copper oxide have been reported in various studies. Investigations have shown that the amount of increase in these enzymes has a direct relationship with the number of damaged cells [21]. It seems that one of the most important reasons for the increase in the activity level of this enzyme in the blood is possible damage to various tissues, including the liver and kidney, and the decrease of this enzyme. It is not due to a disturbance in the transport system of the membrane [18].

In the present study, the change and increase in the levels of biochemical values in the kidney tissues of rabbits that were exposed to experimental poisoning with copper oxide nanoparticles compared to the control group were investigated and clearly observed. Studies have shown that in acute kidney diseases that lead to membrane damage or cell necrosis, the activity of alanine aminotransferase in the blood serum increases significantly [6]. In a study conducted by [3] copper oxide nanoparticles during 72, 48, 24 and 96 hours increased the activity of AST, ALP, LDH and ALT enzymes in carp blood plasma [3]. Also, in another study, copper oxide nanoparticles in concentrations of (7.5 and 15 mg/l) and also Coarse copper oxide particles in concentrations of (25.110 and 5.220 mg/l) increased the activity level of enzymes in the liver of rats and caused a clear and significant disturbance in the liver and kidney function of rats [5]. In a study conducted by [9] on normal mice for 14 days, a significant increase in the level of AST enzyme under the influence of copper sulfate and ALT enzyme under the influence of copper nanoparticles and copper sulfate was observed in the blood plasma of these mice [9]. Tissue marks are another indicators of health evaluation. Many factors can cause tissue damage, which due to the biological and ecological conditions and the surrounding environment, the food industry is susceptible to various types of pollution. Also, another study for seven and 14 days in sub-lethal concentrations of copper oxide nanoparticles in mice showed that this nanoparticle causes a significant decrease in hematocrit, hemoglobin and red blood cells. Researchers believe that oxygen gas transport is intricately related to red blood cells. Therefore, copper oxide nanoparticles reduce the number of red blood cells by creating respiratory restrictions, and with the reduction of red blood cells, other related blood parameters such as MCVMCH, MCHC, as well as hematocrit and hemoglobin are also affected in a study in [16] was performed on mice under the influence of copper oxide nanoparticles, blood parameters such as the number of red blood cells, hemoglobin and hematocrit showed a significant decrease compared to the control, and the amount of MCV, MCH and MCHC indicators

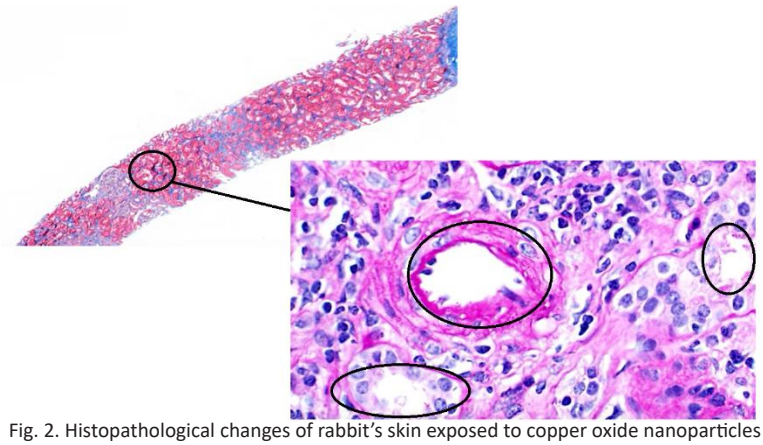

Fig. 2. Histopathological changes of rabbit's skin exposed to copper oxide nanoparticles

also decreased. It is suggested that in the future study, in addition to the biochemical parameters of the kidney, the mentioned parameters should be checked compared to the control. According to the analyzes carried out in the present study, the biochemical parameters of the rabbit compared to the control had a significant increase (P>0.05). In addition to the increase in the amount of copper Nano oxide in the kidney, the kidney tissue has been destroyed and the blood vessels have increased, which is confirmed by the research [19].

CONCLUSION

With the growth of societies, scientific concerns about the desirable features of nanoparticle technology following production and use on a large scale may put human health and the environment at risk. One of the characteristics of nanoparticles to determine their release in living conditions is the small size of these particles, large surface and high reactivity. Therefore, in this research, the effect of Nano-oxides on kidney tissue has been investigated. According to the results obtained from this research, it can be said that the small presence of copper oxide nanoparticles can affect the biochemical parameters of domestic rabbits and further affect other tissue processes and physiology of the kidney. In order to determine the amount of bioaccumulation in kidney tissue, spectrophotometry method was used. The amount of zinc oxide nanoparticles in the kidney tissue in rabbits receiving higher doses was significantly higher than other groups, which indicates more metal accumulation in these doses and damage to the kidney tissue.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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