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RESEARCH ARTICLE

Evaluation of Toxicity of Engine oil Enriched with Copper Nanoparticles and its Impact on Pathology of Intestinal, Liver, Lung and Kidney Tissues

Raham Armand[1], Mohammad Kazem Koohi[2*], Goodarz Sadeghi Hashjin[2], Mehdi Khodabande[2]

[1] Department of Biology, Faculty of Basic Sciences, Behbahan Khatam Alanbia University of Technology, Behbahan, Iran.

[2] Department of Comparative Biosciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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ABSTRACT

Objective(s): Iranian researchers have enriched conventional engine oil with nanoparticles at the Institute of Petroleum Research and hope that this product can replace conventional engine oil due to the improved effects of copper nanoparticles on oils. the purpose of this study was to investigate the pathological effect of engine oil enriched with copper nanoparticles on the rat.

Methods: In this study, 72 female rat were randomly divided into 8 experimental groups, and three treatment groups (repeated) oral doses of 2000 mg /kg and 5000 mg /kg engine oil containing and without copper nanoparticles in three treatment groups at 30 minutes, 4 hours and 24 hours.

Results: In treatment group A and B, oral dose of 5000 mg/kg engine oil containing and without copper nanoparticles showed a significant positive correlation with lethality at 30 minutes, 4 hours and 24 hours. p <0.001). Also in treatment group C, oral dose of 5000 mg/kg engine oil without copper nano particle showed a positive and significant correlation with lethality in 24 hours (p<0.001). Severe hypertension in the cortex and medulla of the kidney, severe hepatocyte swelling, severe hyperemia in the alveolar wall, anaplasmosis like changes and changes in the depth of the intestinal crypts, Were the most important observations of the pathological sections of different tissues at the engine dose of mg /kg. Contains copper nanoparticles.

Conclusions: The results of this study showed that the toxic effects of engine oil containing copper nanoparticles were no more than those of engine oil without copper nanoparticles.

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INTRODUCTION

Nanotechnology is a field of applied sciences that focuses on the design, production, identification and application of Nanoparticles and components. Advances in nanotechnology lead to the improvement of their applications and applications in human life [1]. All materials, regardless of their composition, shrink to less than 100 nm with new properties including improved optical properties, reduced melting point, increased tensile strength, increased catalytic properties, increased magnetic properties, and decreased electrical resistance [2]. Copper nanoparticles, are one of the metal nanoparticles produced, is now commercially available [3,4] and their use in domestic and

* Corresponding Author Email: *mkkoohi@ut.ac.ir*

industrial processes has increased [2]. Copper nanoparticles have recently been widely used as additives in oils, plastics / polymers, metal coatings and inks [3, 4]. Many nanoparticles are at the surface of nanoparticles, so the atoms are more likely to collide, so the material's reactivity also increases, can be present in airborne particles and And they can cause toxicity various ways [5]. In addition, nano materials can be used as catalysts because the nanostructure will increase the amount of voids compared to the usual state. Therefore, nanometer-sized catalysts can be used at lower temperatures [6]. The mechanical properties of nanoparticles are improve with decreasing size [3, 4], the small size of nanoparticles permits crossing the cell membrane in organelles such as mitochondria [7]. According to studies of adverse effects in human cells exposed to nanoparticles, they may be due to the production of free radicals and reactive oxygen species that cause intracellular damage [8 - 10]. This is mainly due to a decrease in the amount of clearance and elimination, which results in a longer stay in tissue and an increase in toxicity [7]. There is currently limited understanding of the adverse effects of nanoparticles in nature and wildlife, so further studies on the toxicity of nanoparticles to onshore animals are needed [8-10]. Due to the ability of nanoparticles to interact with biological agents, the mechanism of interaction between nanoparticles and living systems has certain complexities that, due to the operating environment, result from changes in their surface properties [7]. Currently, nanoparticles that unwittingly arise through combustion processes to generate energy or in cars, mechanical corrosion processes, or conventional industrial processes are more than industrial nanoparticles and affect human environment and life [11]. One of the easiest and most important ways for nanoparticles to enter the human body is the gastrointestinal tract [12]. Another way in which nanoparticles enter the body is by inhalation, which in turn causes many organ involvement. The chemical nature and the electrical charge of the nanoparticles are also other determinants of their risk of inhalation [11]. The intestinal borders in turn are composed of micropores, which provide an area of about 200 square meters for food.

Initially, the particles were thought to pass through the intestinal lymphatic tissues (tissues containing M-cells). Upon further study, scientists found that not only nanoparticles can pass through the intestinal lymphoid tissue, but they can also pass through the normal intestinal tissue. Surface charge, ligand binding, or coating with surfactants are effective in selecting the site and target of particle adsorption [12]. In addition to commercial and industrial [13] applications, copper nanoparticles have an important characteristic that has been of interest to researchers, namely the lubricating properties of copper nanoparticles mixed with the lubricants used in the lubrication industry. Due to the healing effects of copper nanoparticles, they are added to the lubricating oils as an additive to reduce friction and repair the damaged surface at the friction position [14]. Studies of the effect of carbon nanoparticles and titanium oxide with sizes between 1–2 nm on rat have shown that it lowers the defensive power in their lungs, and over time may cause respiratory diseases such as bronchitis or even bronchitis. Cancer becomes lung [10]. In a report by Liu et al., Copper nanoparticles (30 to 120 nm in diameter and 50 nm in diameter) with an oxide coating (used to protect the nanoparticles at ambient and high temperatures without reacting with other oil components) were used. . This oil was 0.1% Wt with a specificity of SN 500 with nano-copper particles content [14]. Domestic researchers at the Oil Industry Research Institute have enriched conventional engine oil with Nanoparticles. With the widespread production of nano-products, there is a pressing need to examine their potential toxic effects on the human body and the environment, and it is still unclear what environmental effects such a compound may have on conventional engine oil. ? And its toxicity in various animal species has not been determined. The present study, therefore, pathologically investigates the acute toxicity of cutaneous engine oil containing copper nanoparticles on animal models of mice. This type of study is necessary to get permission to use new products such as nano products. Therefore, the present design has very important practical aspects.

MATERIALS AND METHODS

Animals

Young, healthy and adult rat were used in this study. First, it was determined that the materials were not pregnant. Animals were 8-12 weeks of age, weighing in the range of 20%; total mean weight of animals (animals ranged between 25-30 g). And the female rat is typically based on literature reviews in LD50 experiments. Animals were kept at room temperature (22-23 ° C) with relative humidity of 70%. Exposure was artificially provided with 12-12

hours of light-darkness. The animals had free access to commercial food and municipal drinking water. Keeping them in a cage required the minimum standard space required.

Dosage Preparation and Engine oil Preparation

The maximum amount of fluid that can be administered at a time depends on the size of the test animal. At this stage, because the engine oil was used for toxicity assessment, and is liquid, the same main oil was used for the toxicity assessment without dilution. The engine oils used were obtained from the Oil Industry Research Institute of the Ministry of Petroleum.

Normal fresh engine oil (no copper nanoparticles)

New engine oil containing copper nanoparticles

Normal working engine oil (8000 km)

Working oil containing nano-copper particles (distance of 8000 km)

Study Design

In this study, 72 female rat were randomly divided into 8 groups ((in 3 different replicates (treatment group) and 3 replicates)). Animals were deprived of food prior to administration. Materials were prepared as a single dose before administration and administered using a suitable cannula.

Experimental group 1: Oral dose of 2000 mg/kg engine oil containing copper nanoparticles in three treatment groups at 30 min, 4 h and 24 h.

Experimental group 2: Oral dose of 2000 mg/kg engine oil without copper nanoparticles in three treatment groups at 30 min, 4 h and 24 h.

Experimental group 3: Oral dose of 5000 mg/kg engine oil containing copper nanoparticles in three treatment groups at 30 min, 4 h and 24 h.

Experimental group 4: Oral dose of 5000 mg/kg engine oil without copper nanoparticles in three treatment groups at 30 min, 4 h and 24 h.

Experimental group 5: Oral dose of 2000 mg/kg engine oil containing copper nanoparticles in three treatment groups at 30 min, 4 h and 24 h.

Experimental group 6: Oral dose of 2000 mg/kg engine oil without copper nanoparticles in three treatment groups at 30 min, 4 h and 24 h.

Experimental group 7: Oral dose of 5,000 mg/kg engine oil containing copper nanoparticles in three treatment groups at 30 min, 4 h and 24 h.

Experimental group 8: Oral dose of 5000 mg/kg engine oil without copper nanoparticles in three treatment groups at 30 min, 4 h and 24 h.

Single animal data were collected. For each experimental group, the number of animals used, the number of animals that showed toxic symptoms, and the number of animals that died during the experiment were shown.

Preparation of tissue sections

After blood sampling, the liver, kidney, intestine, and lungs of the animals were removed, and washed with saline buffer for histopathological and morphological examinations, a section of liver, kidney, intestine, and lung were cut. They were fixed and fixed in 10% formalin and prepared for hematoxylin-eosin (H&E) staining.

Data analysis

The results were analyzed using ANOVA and Duncan tests and the differences between groups were statistically evaluated ($P<0.05$). The relationship between the different doses and concentrations used was determined by the correlation coefficient. This article has a code of conduct number 7506023/6/4.

RESULTS

In treatment group A and B, 5000 mg/kg engine oil containing copper nanoparticles with and without copper nanoparticles showed a positive and significant correlation with lethality at 30 minutes, 4 hours and 24 hours ($p<0.001$)

In treatment group C, the concentration of 5000 mg/kg engine oil containing copper nanoparticles was negatively correlated with lethality at 30 min, 4 h and 24 h and was not significant. Also, there was a negative correlation between the concentration of 5000 mg / kg of engine oil without copper nanoparticles and the tensile strength at 30 minutes and 4 hours.

In group C, 5000 mg/kg engine oil without copper nanoparticles showed a positive and significant correlation with lethality over 24 hours ($p<0.001$) (Table 1).

At a dose of 2000 mg/kg in engine oil containing nano-copper, without nano-copper, as well as in engine oil containing nano-copper, and without nano-copper in 3 different repetitions, the lethal effects in rat at times No different observed. Also in the engine oil containing copper nanoparticles and without copper nanoparticles at dose of 5000 mg/kg, there was a negative correlation with lethality at 30 min, 4 h and 24 h and was not significant and no lethal effect was observed.

Table 1. Oral dose of 5000 mg / kg engine oil containing and without copper nanoparticles in three treatment groups at 30 minutes, 4 hours and 24 hours.

	Oral dose of 5000 mg/kg engine oil containing copper nanoparticles			Oral dose of 5000 mg/kg engine oil without copper nanoparticles		
	30minutes	4h	24h	30minutes	4h	24h
Treatment group A	Live = 2 Dead = 1	Live = 2 Dead = 1	Live = 2 Dead = 1	Live = 2 Dead = 1	Live = 2 Dead = 1	Live = 2 Dead = 1
Treatment group B	Live = 2 Dead = 1	Live = 2 Dead = 1	Live = 2 Dead = 1	Live = 2 Dead = 1	Live = 2 Dead = 1	Live = 2 Dead = 1
Treatment group C	Live = 3 Dead = 0	Live = 3 Dead = 0	Live = 3 Dead = 0	Live = 3 Dead = 0	Live = 3 Dead = 0	Live = 2 Dead = 1

■Positive and significant correlation ($p<0.001$) between 5000 mg/kg engine oil and lethality

□Negative correlation between 5000 mg/kg engine oil concentration and lethality over time

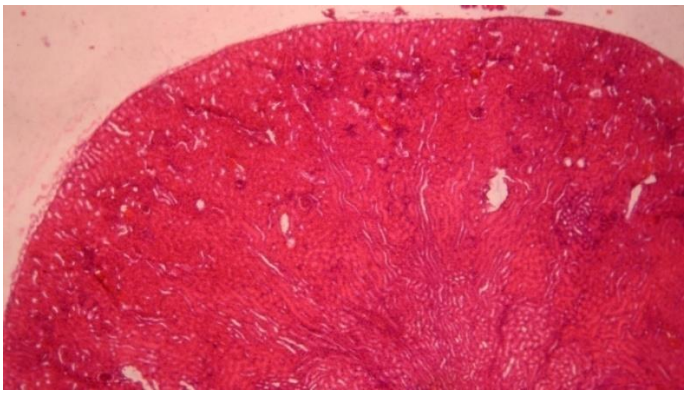

Fig. 1. Severe Kidney tubular swelling (magnification 40) under the influence of engine oil containing 5000 mg/kg copper nanoparticles

Pathological observations of different tissues of rat at a dose of 5000 mg/kg engine oil containing orally administered copper nanoparticles included.

Kidney

Severe hypertension in the cortex and medulla, presence of quasi-profile material inside the tubules indicating glomerular damage, severe tubular cloud swelling in the kidneys, and degeneration of the cells, and central tubular closure, Kidney failure. Ensures (Fig. 1).

Liver

Hepatocytes have become extremely swollen, and this swelling has occurred acutely. None of them have developed a fatty degeneration that is common in the liver and is gradually occurring. The highly vascular and hypertensive reaction indicates involvement of the vascular and blood systems in the liver. The influx of lymphocytes and their focal accumulation within the parenchyma and the connective tissue in the portal space also indicate immune system involvement and sensitivity to the new conditions in the liver, some of which are extensive in the liver. Some have responded milder (Fig. 2).

Lung

The most prominent pathologic change in the lung is very severe hyperemia in the wall of the alveoli. So much so that blood compaction causes the wall of the alveoli to collapse and create a state similar to atelectasis. In addition, there have been cases of intra-alveolar and intra-respiratory bleeding. The presence of macrophages stacked with homocidrin indicates the fact that the process of chronic hemorrhage is present and the presence of fresh blood indicates the persistence of hemorrhage over a period of time. However, there was no edema in these lungs. Neither interstitial nor alveolar type (Fig. 3).

Intestine

The most important pathologic change in the small intestine is changes in the nuclei, changes

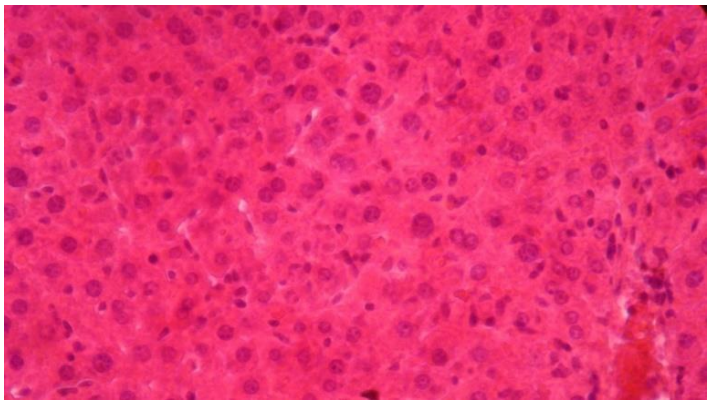

Fig. 2. Severe hepatocyte swelling and nucleated chromatin variations (magnification 400) under the influence of engine oil containing 5000 mg/kg copper nanoparticles.

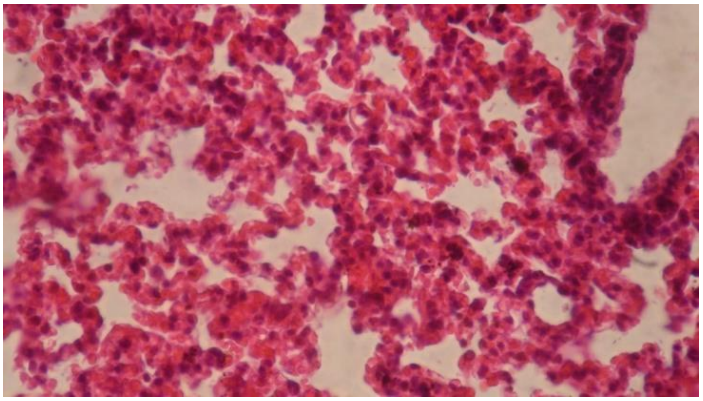

Fig. 3. Severe pulmonary hypertension of the alveolar wall (magnification 400) under the influence of engine oil containing 5000 mg/kg copper nanoparticles.

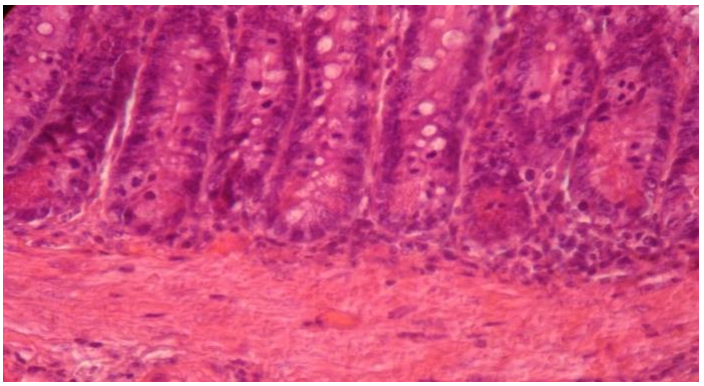

Fig. 4. Lieberkuhn's glands and shapes (400 magnification) under the influence of engine oil containing 5000 mg/kg copper nanoparticles

similar to anaplasm, as well as changes in the depth of the intestinal crypts. It was observed in all intestines of the nano-oral group without exception and with almost the same quality. An increase in the number of abnormal mitotic forms, completely abnormal cell divisions, and the presence of hyaline red droplets were observed within the cytoplasm of cells present in the limbic gland floor (Fig. 4).

DISCUSS

Nanoparticles can enter the gastrointestinal tract directly or through water, food, cosmetics, medicines. Different sizes of particles absorbed through the gastrointestinal tract can have different toxic effects. In the human body, copper plays a role in maintaining homeostasis; excessive consumption of copper causes hemolysis, jaundice, and even death. Scientists' studies show that over-consumption of copper in vivo can have an inducible effect on poisoning activities such as Hepatocirrhosis, It also has altered lipid profile, kidney failure and stimulatory effect on gastrointestinal mucosa. In general, studies performed orally on nanoparticles suggest that nanoparticles exhibit different toxic effects in vivo than larger particles [15–19]. The difference between the copper nanoparticles and the copper microparticles is due to the change in the surface area of the nanoparticles. However, few tests have been conducted on the commercial toxicity of commercially made Nanoparticles, which are studies of nanoparticles neither made from the product nor after use. Regarding engine oil, one of the routines used to market a petroleum product, such as engine oil, is the acute testing of engine oil in rat and rabbits. One of the studies performed in this study was the study of acute oral toxicity of titanium oxide in rat by Wang-j et al. It was observed and also marked changes in serum enzymes [20]. Another study was conducted in 2005, which showed that high-dose micro-zinc can cause severe liver damage to copper nanoparticles, while zinc nanoparticles can cause severe damage to all. This indicates that there is a difference in the activity of the toxicity of the material in different sizes [21]. Also in a study by Chen et al showed that LD50 copper nanoparticles were typically 134 mg/kg and based on OECD 413 mg / kg toxicity in the middle class, in the case of LD50 copper microparticles in the conventional method of 5600 mg / kg. In the OECD method, this value was more than 5000 mg/kg, indicating that the class is practically informal, for the LD50 copper ion for the conventional method 189. 109 mg / kg and in the OECD method, this value was 110 mg/kg. There was a moderate toxicity class [22]. And in line with the results of this study. This study, like any other study, has some drawbacks. In this study, oral doses were studied and it is recommended to evaluate its side effects and compare the results with similar conditions.

CONCLUSION

The results of this study showed that the toxic effects of engine oil containing copper nanoparticles were no more than the engine oil without copper nanoparticles, and the toxic effects of engine oil containing copper nanoparticles with and without copper nanoparticles with and without engine oil. Copper nanoparticles are smaller. But according to the classification in the instruction, both engine oils containing copper nanoparticles and those with or without engine oils containing copper nanoparticles are classified in group 5. That is, their toxic effects are higher than 5000 mg/kg.

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CONFLICT OF INTEREST

None declared.

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