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Effects of *Lavandula angustifolia* Extract and Copper Oxide Nanoparticles (CuO-NP) on Some Biochemical Outcomes in Mouse Blood Tissue

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ABSTRACT

Our study is on the protective effect of lavender oil on oxidative stress caused by copper oxide nanoparticles (CuO NPs). For this purpose, 0.5 mg/kg body weight *L. angustifolia*, 5.0 mg/kg body weight CuO NPs and their mixtures (0.5 mg/kg body weight *L. angustifolia* +5 mg/kg body weight CuO NPs) were administered to mice by gavage method for 14 days. At the end of the time, antioxidant activities such as biochemical parameters (ALT, AST, T. Prot, Cholesterol) in mouse blood tissue and their combined effects on copper accumulation in kidney and liver tissues were investigated in male Swiss albino mice. The study results showed that copper accumulation in liver and kidney tissue increased significantly. While ALT and AST values in blood tissue increased compared to control, significant decreases were observed in T. Protein and cholesterol levels. These results showed us that lavender oil was significantly effective in homeostatic effects against any toxicity in mouse blood tissue.

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Introduction

The production of nanomaterials using chemical techniques is extremely costly, requires complex processes and can lead to the accumulation of toxic chemicals on the surface. This can have adverse effects in medical fields and can pose an environmental hazard. (Rehana et al., 2017).

Despite the promising uses of CuO NPs in healthcare, the biggest concern regarding their application for diagnostic and therapeutic purposes is their toxic effects, which have been reported by scientists working in different vertebrates (especially mammalian cells) and invertebrates. CuO NPs have been reported to induce oxidative stress in living cells by causing overproduction of reactive oxygen species (ROS), resulting in damage to DNA and cell organelles. The size, surface charge and dissolution properties of NPs are the main factors that are effective in causing toxicity of CuO NPs in vitro and in vivo (Naz et al., 2023).

Copper oxide (CuO)/copper (II) oxide/cubic oxide is a semiconducting compound with a monoclinic structure. CuO is the simplest member of the family of copper compounds, exhibiting potentially useful physical properties such as temperature superconductivity, electron correlation effects and spin dynamics (Katwal et al., 2015).

İt is of great importance to develop clean, biocompatible, harmless and environmentally friendly techniques through microorganisms, enzymes and plant extracts in nanotechnology. Among various biosynthetic methods, plant extracts are preferred because they are safe to process, readily available and have a rich range of metabolites. Plant chemicals such as carbohydrates, flavonoids, saponins, proteins and amino acids and terpenoids in plant extracts have a decisive importance in the synthesis of nanoparticles (Rehana et al., 2017).

Lavenders belong to the plant family Labiatae (*Lamiaceae*). Most commercially marketed lavender essential oils are derived from cultivars of two lavenders species, *L. angustifolia* and *L. latifolia*, as well as from lavender hybrids, commonly known as lavandins. Other

lavender species such as *L. stoechas* and *L. dentata* have also been used in folk medicine applications for their antispasmolytic properties (Gilani et al., 2000).

Lavender oil, extracted from the *Lavandula angustifolia*, is a popular essential oil known for its therapeutic properties such as calming and relaxing effects. It has also been found to have antimicrobial, anti-inflammatory and antioxidant properties. In recent years, lavender oil has been investigated for its potential to reduce the harmful effects of various toxic substances, including heavy metals and nanoparticles.

Plant flavonoids have been used as food for many years. These flavonoids are important for health. Since there is no study on the accumulation of *Lavandula angiustifolia* oil with CuO NPs in mouse blood tissue, liver and kidney, this research was needed. When lavender oil is combined with copper nanoparticles, it can counteract oxidative stress induced by CuO NPs and prevent cellular damage. In this study, it was aimed that lavender oil can increase the activity of antioxidant enzymes since it is known to be able to effectively scavenge free radicals. The effects of *Lavandula angustifolia* extract on biochemical parameters in mouse blood tissue induced by CuO NPs, antioxidant activities such as ALT, AST, T. Prot, Cholesterol and copper accumulation in kidney and liver were investigated in male Swiss albino mice.

Materials and Methods

In the first period of our study, August 1, 2023 - February 27, 2024., the necessary literature review studies were conducted. Appropriate literatures were found and the necessary literatures for the study were obtained.

The plant sample to be used in the project was collected during this period. The dried plant samples were dried in the shade and the parts of the dried plant samples were pulverized with the help of a mill. The powdered plant sample was extracted with methanol at a concentration of 10 mg/ml. The extraction process was carried out at 30°C for 24 hours in a heater mixer. The plant extract was stored at 0-4°C for biological activity assays.

We started our experiments in the second period of our study August 1, 2023 - February 27, 2024.

During this period, we purchased the necessary chemicals. After overcoming our difficulties in finding experimental animals, we finished the experimental parts, analyzed and wrote the result reports.

Experimental animals (Swiss Albino Mice) were obtained from Cukurova University, Health Sciences, Experimental Animal Research Center (SABIDAM) and animals were housed in stainless steel cages in a ventilated animal experiment room. Control and experimental mice were maintained at 22 ± 2 °C for 12 h on a light/dark cycle. Temperature and 50% humidity under standard conditions. Twenty-four (24) mice were divided into 4 groups (4 groupsX6 mice) so that the weight of the mice was approximately 25-30 g.

Cukurova University, Health Sciences, Experimental Animal Research Center (SABIDAM) was contacted for the supply of mice required for our study and the necessary interviews for experimental animals and Ethics Committee Reports (31.10.2023; 8/1) of the experiments were obtained.

Copper nanoparticle size

Characterization of copper nanoparticles was performed by Gomez et al., (2011) method. Prepared CuO NP suspensions were dispersed in tap water at concentrations of 100 mg/l. The obtained suspensions were sonicated for 15 min using a bath sonicator (USC500TH, VWR International) immediately before analysis. Then, the samples were analyzed in terms of NP size (z-average hydrodynamic diameter) and surface charge (zeta potential). CuO NPs were also characterized by X-Ray diffraction (XRD). (Size nm <50, Zeta potential mV -16.2±0.4).

Standard rat food (Rat Food, DSA Agrifood Products Inc., Kırıkkale, Turkey) was fed during the experiments.

Plant samples were collected for use in the project. Parts of the plant samples dried in the shade were pulverized with the help of a mill. The powdered plant sample was extracted with methanol at a concentration of 10 mg/ml. The extraction process was carried out at 30°C for 24 hours in a heated mixer. The plant extract was stored at 0-4°C for use in biological activity assays (Tepe et al., 2007). Lavender dose selections selected in the experiment were made based on the ratios of the doses (100 mg/kg body weight) in the study conducted by Torabzadeh et al. (2017).

Mice were administered extracts and CuO NPs by oral gavage (0, 0.5, 5.0 and 0.5+5.0 mg/kg body weight/day) in 100 µL water. After 14 days, mice were dissected under high dose anesthesia (ketasol 10%). Blood was collected with a syringe and glass tubes. Blood samples were centrifuged at 3000 g (Hettich Universal 30 RF, Germany) for 5 minutes (4°C) to separate serum and cells.

Direct spectrophotometric analyses of biochemical effects such as ALT, AST, Total Protein and cholesterol in serum samples were performed at the Seyhan State Hospital Central Laboratory (Modular Roche DPP, Modular Roche Cobas C501, Hitachi Ltd, Tokyo, Japan) (Bergmeyer et al., 1985).

Copper accumulation in liver and kidney tissue was measured with a Perkin Elmer AS 3100 Atomic Absorption device.

An SPSS statistical software (SPSS 17, Chicago, IL) was used to analyze the data. One-way ANOVA test was applied to compare plant extract doses and CuO levels.

Table 1. Experimental groups and their abbreviations (N=6).

Groups	Experimental Groups	Abbreviation
Group I	Control	C
Group II	0.5 mg/kg body weight L. angustifolia	LA
Group III	5.0 mg/kg body weight CuO NPs	Cu
Group IV	0.5 mg/kg body weight L. angustifolia +5 mg/kg body weight CuO NPs	Cu+LA

Results

No deaths were encountered in any group during the experiments. There was no change in the external appearance of the mice. There was no change in the behavior of the mice.

Copper accumulation was observed to increase significantly in the liver and kidney tissue (Table 2.). When the liver and kidney tissue were compared, it was observed that there was more accumulation in the liver compared to the control. Liver tissue is one of the organs where heavy metals such as copper accumulate the most (Hollis et al.,2001; Roberts & Sarkar, 2008). In addition, the liver is the main site of synthesis of detoxification proteins such as metallothionein (Çoğun & Kargın, 2004). In our study, it was observed that copper nanoparticles were increased in the liver compared to the control. In similar studies, (Wang et al., 2014) observed that copper had bioaccumulation and toxic effects on the hepatocellular structure in mice. Similarly, (Liu et al., 2020) observed that copper induced oxidative stress and apoptosis in the liver in mice.

Copper (Cu) is an essential trace metal, the third most abundant essential transition metal in humans. Copper is a fundamental element for the homeostasis of the body. One of the important organs where copper accumulates is in the kidney tissue of mice (Kurasaki et al.,1998). In addition, copper accumulates significantly by binding to metal binding sites called metallothionein in the mouse kidney (Kurasaki et al.,1998). In our study, it is thought that copper nanoparticles accumulate in the kidney tissue by binding to metallothioneins. Similarly, (Petering et al., 1984) observed that copper binds to methylothionein in the mouse kidney in their study.

Copper (Cu) is one of the most useful elements in the field of health due to its numerous anti-inflammatory, anticancer, analgesic and antimicrobial effects (Sarayaadevi et al.,2014). In recent years, it has been proven that Copper oxide nanoparticles (CuO NPs) are highly reactive due to their high surface-to-volume ratio and can easily interact with other particles, leading to a wide range of biological activities (Naika et al.,2015). Liver damage is characterized by increased serum alanine transaminase (ALT) and aspartate transaminase (AST) levels (Xuan et al.,2015). Nowadays, one of the main criteria for determining liver damage is the measurement of serum levels of enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Fallahi et al.,2017). Plant structures contain secondary metabolites such as alkaloids, volatile oils, glycosides, steroids, saponins, flavonoids, tannins, phenols, colorants and resins. Their interactions with toxic agents have significant effects on alanine transaminase (ALT) and aspartate transaminase (AST) levels in blood tissue.

In our study, when CuO NPs was treated alone, an increase in ALT and AST enzyme levels was observed compared to the control group. Compared to this situation, ALT and AST enzyme levels decreased in the combination of CuO NPs and *Lavandula angustifolia* oil.

When other parameters were examined, a decrease in cholesterol and total protein levels was observed when CuO NPs were treated alone compared to the control group. Compared to this case, a more significant decrease in cholesterol and total protein levels was observed in the combination of CuO NPs and *Lavandula angistiufolia* oil.

Discussion

In a study, it was observed that *Origanum vulgare* (thyme) extract had protective effects against liver toxicity in mice (Habibi et al., 2015). In our study, it was observed that *Lavandula angustifolia* oil was effective in blood parameters such as ALT and AST in copper toxicity. In a similar study, (Jumaily et al., 2023) observed that there were decreases in ALT and AST levels in the effectiveness of dandelion root extract to reduce the toxic effect of copper sulfate in male albino mice.

Cholesterol is a type of lipid found in living blood and produced naturally by all cells, especially in the liver. The body also needs a certain amount of cholesterol to make certain hormones and vitamin D. In our study, cholesterol levels in mouse blood showed a decrease in the effect of copper oxide nanoparticles and a further decrease was observed in the mixture of *Lavandula angustifolia* and Copper Oxide. In a similar study by (Prohaska et al., 1985), it was observed that serum cholesterol levels did not increase. This is thought to be related to the metabolic copper effect (Klevay, 1977). Lavender's properties of antioxidant and free radicals absorption can be effective in reducing cholesterol (Ramchoun et al., 2009).

Table 2. Copper levels in liver and kidney tissues of mice on the 14 days after lavender, copper nanoparticles and their mixtures (Letters a and b in the tables indicate concentration differences SNK; $p<0.05$).

	Liver	Kidney
C	5.80±0.15 α	7.56±0.22 α
LA	5.75±0.16 α	7.66±0.15 α
Cu	7.25±0.12 β	8.26±0.24 β
Cu+LA	7.50±0.22 β	8.90±0.20 β

Table 3. ALT, AST, Cholesterol and Total Protein levels of lavender, copper nanoparticles and their mixtures in mice on the 14 days (Letters a, b, c and d in the tables indicate concentration differences SNK; $p<0.05$).

	ALT (u/l)	AST (u/l)	Cholesterol (mg/dL)	Total Protein (g/dL)
C	36.1±2.25 α	112.2±3.2 α	73.15±2.23 α	61.2±1.12 α
LA	43.2±2.23 β	174.1±4.2 β	70.33±1.12 α	54.5±2.22 β
Cu	58.2±2.16 χ	340.1±5.2 χ	62.45±2.24 β	26.1±2.44 χ
Cu+LA	49.2±2.11 δ	268.4±4.4 δ	38.35±1.22 χ	20.4±2.16 δ

Proteins have an important place among the basic building blocks in living things. Especially with the tasks they take, they allow vital values to remain at a healthy point. Total protein measures the amount of proteins present in the blood plasma. In our study, significant decreases were observed in mouse blood tissue under the effect of copper nanoparticles and lavender mixtures. In a similar study, it was observed that the effect of copper on protein levels in mouse blood tissue (Broderius & Prohaska, 2009).

Conclusion

In conclusion, our study showed significant changes in the effect of copper oxide nanoparticles on blood tissue biochemical parameters (ALT, AST, cholesterol and total protein) levels. It was also observed that CuO nanoparticles caused significant accumulation in mouse liver and kidney tissues. This accumulation is thought to potentially lead to toxic effects. The accumulation mechanisms and long-term effects of CuO nanoparticles after different exposure routes in the body should be investigated in detail. The development of innovative approaches to reduce or eliminate the accumulation of CuO nanoparticles in biological systems is thought to be a research area for safe applications of nanotechnology. Including more toxicity studies using other plants with antioxidant properties, such as *Lavandula angustifolia*, will help clarify the bioaccumulation mechanism.

Declarations

Ethical Approval Certificate

In order to obtain the mice required for our study, we contacted Cukurova University, Health Sciences, Experimental Animal Research Center (SABİDAM) and the necessary interviews for the experimental animals and the Ethics Committee Reports of the experiments were obtained (31.10.2023; 8/1).

Author Contribution Statement

Please indicate how each author contributed to this work and at what stage. For example:

S.Y.: Data collection, investigation, formal analysis, and writing the original draft, project administration, methodology

Z.Ş.: Data collection and investigation, conceptualization, methodology, review and editing

H.Y.Ç.: Supervision, conceptualization, review and editing, formal analysis, and writing the original draft

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Conflict of Interest

The authors declare no conflict of interest.

Abbreviations and Units

Kilogram: kg, Centigrade: C, Gram: g, Milligram: mg, Hours: h, Microliter: µL, Deciliter: dl

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