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MITIGATION OF COPPER-INDUCED TOXICITY IN WISTAR RATS' SERUM BIOCHEMICAL PROFILE BY CANNABIS LEAF EXTRACT

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ABSTRACT

Copper serves as an integral part of specialized cuproproteins responsible for normal growth and development of the body, and excess accumulation in the body has a detrimental effect. Anthropogenic influences and an increased number of vehicles coupled with intensive urban and rural infrastructure result in the accumulation of heavy metals in the soil and plants, which are subsequently transmitted to humans via the food chain. The study was designed to provide positive effects of cannabis, establish a means of preventing copper exposure, and devise a means of preventing them. The aim of the study was to determine the therapeutic effect of cannabis leaf extract on copper-induced toxicity and serum biochemical profile changes. The experimental study was carried out using 20 Wistar rats allocated into 4 groups containing 5 rats each: group A (negative control), group B (copper only), group C (cannabis only), and group D (copper then treated with cannabis). The results showed decreased serum electrolytes (urea, bicarbonate, creatinine, total protein, albumin, bilirubin, AST, and ALP) and increased serum ALT and erythropoietin, which were later reversed after treatment with cannabis. In conclusion, cannabis leaf extract has therapeutic effects on copper toxicity.

Keywords: Cannabis, Copper, Serum Biochemistry, Wistar Rat

INTRODUCTION

A vital microelement, copper is involved in numerous processes that lead to normal growth and development. It is also a crucial component of specific cuproproteins like tyrosinase, cytochrome c oxidase, dopamine β-hydroxylase, superoxide dismutase, and ceruloplasmin (Mladenovic and Paunovic, 2014). It is also involved in maintaining some normal physiological and biochemical functions in the animal's body (Tang *et al.*, 2019). It is also an important trace element that serves as an integral component of many proteins and enzymes needed in a wide range of metabolic activities, including an effective immune response (Mohammed, 2014). Natural environments can result in copper poisoning or deficiency, which can cause pathological lesions, decreased animal productivity, and finally the animal's mortality (Nederbragt et al., 1984).

While copper is a vital micronutrient that is typically under effective homeostatic control, excessive dietary intakes can be harmful. The risk of copper toxicity varies depending on a number of factors, such as the species. For example, sheep may be more susceptible to copper toxicity because they cannot increase their biliary excretion of copper in response to increased intakes, but pigs are so tolerant of copper that they were once fed diets containing 250 mg Cu/kg as a growth stimulant. Long Evans Cinnamon (LEC) rats are susceptible to copper poisoning because they lack a specific copper transporter protein, especially in the liver; age, likely as a result of the immaturity of the biliary excretory mechanisms in younger animals and the high absorption efficiency of copper; and diet, which may contain dietary antagonists of copper metabolism, such as sulfur and other trace metals, as well as other hepatotoxins or protective factors (Bremner, 1998). Redox-active metals, like copper, are well known for their ability to induce oxidative stress by elevating the generation of reactive oxygen species (ROS). This, in turn, results in the peroxidative degradation of polyunsaturated fatty acids in membrane lipids, damaging biomolecules in the process (Mladenovic and Paunovic, 2014). Intentionally high copper uptakes can induce liver and kidney damage, as well as even death. Metal fume fever, caused by industrial exposure to copper fumes, dust, or mists, can produce atrophic alterations in nasal mucous membranes (Buck and Sharma, 1969; Osredkar and Sustar, 2011). It happens when animals consume too much copper salts. This can happen in a number of ways, including grazing right after fertilization, pastures planted in high-copper soils, animal feed treated with antifungal medications that contain copper, pastures tainted by foundry smoke, and the feeding of mineral mixes that contain too much copper. Acute or chronic poisoning of this kind is possible (Reis et al., 2010).

Copper storage disease is characterized by liver damage. The toxic ability of copper, derived from its ability to bind to sulfhydryl groups, nucleic acids, and tubulin, leads to impairment of cellular functions such as protein synthesis, enzyme activity, and intracellular transport, as demonstrated in vitro studies (Nederbragt *et al*., 1984). The increased intracellular level of copper leads to the dissociation of hydrogen peroxide, yielding hydroxide ions, which initiate phospholipid peroxidation and protein oxidation (Musacco-Sebio *et al*., 2017). Hemolysis is a potentially catastrophic consequence of copper buildup in the liver. A higher amount of blood copper indicates the start of this stage. While stress conditions may precede the production of this copper, it is widely believed that the liver releases it. The cause of its release is unknown. Because their membranes are high in unsaturated fatty acids because they are continually exposed to reactive oxygen species, erythrocytes are more vulnerable to oxidative damage than other types of cells. Increased blood copper causes hemolysis as a result of oxyhemoglobin oxidation, methemoglobin synthesis, erythrocyte destruction, and the formation of Heinz bodies (Mladenovic and Paunovic, 2014).

The degree of hemolysis in rats is determined by the duration of exposure of their red blood cells to an increasing concentration of copper, rather than the height of the blood

copper content, as Niederbragt et al.'s 1984 study. This was observed after injecting rats with a high dose of copper.

Dried leaves and flowers of cannabis plants containing Δ9-tetrahydrocannabinol (THC) at varying concentrations are referred to as cannabis, also known as Wiwi, Ganja, Marijuana, and Helm (Osadolor and Mathias, 2010). It is described as "the flowering of fruiting tops of the Cannabis plant (of the genus Cannabis)" in the 1961 United Nations Single Convention on Narcotic Drugs. Cannabis and its constituent phytocanabinoids are increasingly being used therapeutically, and this is drawing major interest from the medical community and public. However, the field of medicinal cannabis is still relatively new to clinical pharmacology (Dryburgh et, 2018).

A diverse range of compounds, including Δ9-tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBND), are found in cannabis spp. Up to 568 unique molecules have been identified in cannabis to date. While some cannabinoids, like cannabidiol (CBD), are nonpsychoactive, they influence the body through a variety of receptors, such as adrenergic receptors, cannabinoid receptors (CB1 and CB2), and several others. (Lewis et al., 2017). The most potent psychoactive ingredient in cannabis, tetrahydrocannabinol, has been isolated, synthesized, and extensively studied; other plant cannabinoids, such as cannabinol and cannabidiol, have effects that are additive, synergistic, or antagonistic to tetrahydrocannabinol, and they may alter its action when smoked; synthetic cannabinoids, like nabilone, are also available for research and therapeutic purposes (Ashton, 2001).

According to Lewis et al. (2017), plant species that yield cannabis and its derivatives are either prohibited or regulated worldwide, and their possession is prohibited in many nations due to a unique class of compounds known as phytocannabinoids. There is an extensive literature on the effects of cannabis, with human beings being the topic of many of the older investigations (Mendelson, 1976). The prevalence, correlates, and effects of cannabis use have been the subject of increasing research in recent decades. This has led to public discussions regarding the benefits and drawbacks of legalizing or decriminalizing cannabis, as well as rising rates of cannabis use in many societies (Fergusson et al., 2003). Due to a lack of epidemiological research and differences over the interpretation of the scant epidemiological and laboratory evidence, the health effects of cannabis use—especially long-term use—remain unclear (Hall and Solowij, 1998).

The laboratory rat belongs to the order Rodentia and family Muridae. They are widely used in toxicological, nutritional, genetics, and environmental studies due to their short life span, short gestation length, and smaller size, which make them preferable animals in conducting research due to their ease of housing and caring (Hrapkiewicz *et al.*, 2013).

Anthropogenic influences such as mining, irrigation, pesticide use, and chemical fertilizers bring about environmental contamination by heavy metals. The increase in the number of vehicles coupled with the intensive construction of urban and rural infrastructure results in the accumulation of heavy metals in the soil and plants, which are then transmitted to humans and animals, causing toxic, allergic, and carcinogenic effects on certain organs. The aim of this study is to determine the therapeutic effect of cannabis on copper-induced toxicity in Wistar rats serum biochemical profiles. The study will provide information on the positive effects of cannabis, contrary to many people's perceptions with regards to the abusive notions about cannabis plant products. The study will help in establishing possible means of exposure to copper and devising means of preventing them.

MATERIALS AND METHODS

Materials

Cannabis leaves extract, Wistar rats, EDTA bottles, weighing balance, scalpel blade, scissors, cotton wool, forceps, hand gloves, cages, distilled water, chloroform, microscope, glass slides, capillary tube, plasticine, Neubert counting chamber, RBC pipette, WBC pipette, micro hematocrit reader, Turk solution, heyem solution, drabkin solution, cover slip, methanol, beaker, measuring cylinder, copper (II) oxide, syringes, and hematocrit centrifuge.

Study Area

The study was carried out in the lab of Usmanu Danfodiyo University's Department of Veterinary Physiology and Biochemistry at the Faculty of Veterinary Medicine in Sokoto, Sokoto State. Sokoto State occupies 28,232.37 square kilometers of territory and is located in Nigeria's extreme northwest. The State lies between latitudes 4º and 6º North and longitudes 11º 30~ to 13º 50~ East. Its borders are as follows: Kebbi State to the south and west, Zamfara State to the east, and the Niger Republic to the north (ICT Directorate, Sokoto State Government, 2023).

Study Design

The random sampling technique was used in an experimental investigation. Twenty Wistar rats, five of each grouping, were randomly assigned to be male or female and weigh between 130 and 250g. With only food and water, the first group acted as the control. For five days, 200 mg/kg of copper II oxide was administered to the second group as a positive control. The fourth group merely received 250 mg/kg of cannabis for ten days, while the third group was treated with 200 mg/kg of copper II oxide for five days, followed by a ten-day period of cannabis treatment.

Experimental Animals

Twenty adult Wister rats, both male and female, weighing between 130 and 250 grams, were acquired from the animal house at Usmanu Danfodiyo University, Sokoto, Sokoto; the department is part of the faculty of pharmaceutical sciences. They were kept in a cage with good ventilation. The rodents were fed regular rat chow and had unlimited access to tab water. Prior to the experimental period, they spent two weeks acclimating (Dryburgh et al., 2018). The procedures that involved the care and use of animals were carried out in compliance with the National Institute of Health's (NIH) recommendations (NRC, 1996). The Faculty Animal Research and Ethics Committee (FAREC) of the Usmanu Danfodiyo University, Sokoto's Faculty of Veterinary Medicine was consulted for ethical permission.

Plant Material and Extraction

The leaves of cannabis were sourced from Sokoto, Nigeria. Using a mortar and pestle, the leaves were ground into a powder and then soaked in 800 milliliters of methanol and 200 milliliters of distilled water. The mixture was then stored for seven days at room temperature and dust-free. After sieving it through soft cotton cloths, it was allowed to partially evaporate the ethanol for seven days at ambient temperature. It was then heated to 50°C in a rotary evaporator and freeze-dried. The aqueous extract obtained was represented by the yield of the freeze-dried sample.

Exposure to Copper II Oxide and Cannabis Leaf Extract
A dosage of 200 mg/kg/rat of copper (II) oxide was given orally for 5 days throughout the experimental period. A dosage of 250 mg/kg/rat of cannabis leaf extract was given orally for 10 days.

Determination of Serum Biochemistry
A cardiac puncture was used to collect the blood sample, which was then placed into a plain sample bottle. The serum was then separated for biochemistry analysis to determine the levels of electrolytes, urea, creatinine, total protein, albumin, bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and erythropoietin. The centrifuge was run for five minutes at 3000 revolutions per minute.

Statistical Analysis
The data obtained were analyzed using InVivoStat software version 4.2.0, and the result was expressed as the mean plus or minus (±) standard error of means (SEM). The overall treatment effects were assessed using the non-parametric Kruskal-Wallis test. and p values less than 0.05 were considered significant.

RESULTS AND DISCUSSION
The result of this study in relation to the liver and kidney functions test, as shown in Table 1. There was a statistically significant decrease ($p < 0.05$) in creatinine in general (group A) when compared with the group given copper only (group B), the group given cannabis only (group C), and the group treated with copper and then cannabis (group D). There was a statistically significant ($p < 0.05$) increase in total protein in the general group (group A) when compared with the group given copper only (group B), the group given cannabis only (group C), and the group treated with copper and then cannabis (group D). There was a statistically significant ($p < 0.05$) increase in albumin in the general group (group A) when compared with the group given copper only (group B), the group given cannabis only (group C), and the group treated with copper and then cannabis (group D). There was a statistically significant ($p < 0.05$) decrease in total bilirubin in the general group (group A) when compared with the group given copper only (group B), the group given cannabis only (group C), and the group treated with copper and then cannabis (group D). There was a statistically significant ($p < 0.05$) decrease in AST in the general group (group A) when compared with the group given copper only (group B), the group given cannabis only (group C), and the group treated with copper and then cannabis (group D). There was a statistically significant ($p < 0.05$) decrease in ALT in the general group (group A) when compared with the group given copper only (group B) and the group given cannabis only (group C), but an increase when compared with the group treated with copper and then cannabis (group D). There was a statistically significant ($p < 0.05$) decrease in ALP in the general group (group A) when compared with the group given copper only (group B), the group given cannabis only (group C), and the group treated with copper and then cannabis (group D). There was a statistically significant ($p < 0.05$) decrease in erythropoietin in the general group (group A) when compared with the group given copper only (group B), but an increase when compared with the group given cannabis only (group C) and the group treated with copper and then cannabis (group D).

Table 1: Effect of cannabis leaf extract on copper-induced serum chemistry changes of liver and kidney functions test in Wistar rats (N = 20)

Parameters	A	B	C	D
CREA (mg/dl)	0.90 ± 0.15	1.05 ± 0.19^{ac}	1.00 ± 0.19^{a}	1.25 ± 0.15^{a}
TP(g/dl)	7.50 ± 0.24	7.03 ± 0.21^{bc}	7.30 ± 0.20^{b}	7.15 ± 0.45^{b}
ALB(g/dl)	4.10 ± 0.27	4.05 ± 0.14^{bc}	4.10 ± 0.18^{b}	4.20 ± 0.00^{b}
BL(mg/dl)	0.20 ± 0.06	0.24 ± 0.06^{ac}	0.24 ± 0.06^{a}	0.24 ± 0.14^{a}
AST(U/L)	45.00 ± 1.16	50.00 ± 5.40^{ac}	54.25 ± 7.32^{a}	48.50 ± 10.50^{a}
ALT(U/L)	8.67 ± 1.45	10.25 ± 1.65^{ad}	10.25 ± 0.63^{a}	8.00 ± 1.00^{b}
ALP(U/L)	607.00 ± 5.01	612.75 ± 94.69^{ac}	611.00 ± 96.23^{a}	746.50 ± 144.50^{a}
ERY(pg/ml)	29.20 ± 11.13	38.13 ± 3.49^{ad}	31.93 ± 5.28^{b}	31.97 ± 2.40^{b}

Keys: ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), ALP (Alkaline Phosphatase), TP (Total Protein), CREA (Creatinine), ALB (Albumin), ERY (Erythropoietin), BL (Bilirubin). Values with different superscript showed statistical difference [increase (^{a,d}) decrease (^{b,c})].

Discussion
This study showed an increase in creatinine concentration, which is consistent with the work of Tang *et al.* (2019), who reported an increase in creatinine due to kidney injury caused by nano copper. This study shows a reduction in total protein, albumin, and is consistent with the work of Tang *et al.* (2019), who reported that the liver is the main organ for protein synthesis and nano copper causes liver dysfunction, which leads to a significant decrease in total protein. There was an increase in bilirubin concentration, which is a result of hemolysis or liver damage, denoted by increased activity of serum transaminases. The AST and ALT activity was significantly increased in this study, which is consistent with the work of Mladenovic and Paunovic (2014), who reported that copper treatment increased transaminase (AST and ALT) activity in the serum due to the distortion of the functional integrity of the hepatocyte cell membrane and the release of this enzyme in the serum. The ALP was significantly increasing following copper and cannabis administration, which is consistent with the work of Tang *et al.* (2019), who reported that high doses of copper nanoparticles cause a significant increase in ALP. Erythropoietin concentration was also increased in this study following administration of copper, which may be due to hemolysis, but decreased following administration of cannabis and copper. There was a decrease in creatinine, total protein, albumin, bilirubin, AST, and ALP and an increase in ALT and erythropoietin in the group given copper only (group B), but they increased in the group treated with copper and then cannabis (group D), except for ALT and erythropoietin, which were decreased, which indicated that cannabis has ameliorated the effects of copper toxicity on the liver and kidney.

CONCLUSION
Based on the findings of this study, it was experimentally shown that copper has toxic effects on the liver and kidney,

but cannabis has reversed the effects caused by copper on the kidney due to a decrease in erythropoietin and increased electrolyte concentration in the serum, as well as the effect of copper on the liver due to an increase in total protein, albumin, and a decrease in ALT concentration in the serum. Cannabis can also cause other nonspecific effects on the body, as denoted by the increased levels of AST, ALP, and creatinine in the serum. Despite the therapeutic effect of cannabis on copper toxicity, it should not be used to treat the toxicity because it also causes damage to the liver and kidney. Iron pipes used in municipal water supplies should be replaced with plastic ones, as they serve as a source of copper exposure due to corrosion or sipping of copper accumulated into the pipes when the pipes are leaking.

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