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Research Article

Therapeutic Effects of 80% Methanolic Cannabis Sativa Leaf Extract on Copper (II) Oxide-Induced Hepatorenal Toxicity in Wistar Rats

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Abstract

This study aimed to investigate the protective effect of 80% methanolic *Cannabis sativa* leaf extract against copper (II) oxide (CuO)-induced hepatorenal toxicity in Wistar rats. Acute toxicity studies were conducted to determine the median lethal dose (LD_{50}) of the crude extract and CuO, followed by an in vivo assessment of the protective effect of the extract in rats exposed to CuO. The crude *C. sativa* leaf extract exhibited relatively low acute toxicity, with an LD_{50} of 676.08 mg/kg, compared with 45.71 mg/kg for CuO, indicating greater acute toxicity of CuO. CuO exposure produced significant alterations in selected hepatic and renal biochemical parameters compared with the normal control group. Administration of the 80% methanolic *C. sativa* leaf extract following CuO exposure significantly ameliorated these alterations, with significant differences ($P < 0.05$) observed among the experimental groups. The observed changes in hepatic and renal biomarkers suggest that CuO exposure may impair normal hepatorenal function, whereas the improvement following extract administration indicates a potential protective effect of *C. sativa*. These findings suggest that 80% methanolic *C. sativa* leaf extract possesses ameliorative properties against CuO-induced hepatorenal toxicity in Wistar rats. The extract may therefore represent a potential source of bioactive compounds for mitigating heavy-metal-induced hepatic and renal injury. Further studies are warranted to identify the active phytochemical constituents and elucidate the molecular mechanisms underlying the observed protective effects.

Keywords

Liver, Kidney, CuO, Cannabis, Rats

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1. Introduction

Cannabis sativa is a medicinal plant that has been used traditionally for centuries [1]. Its leaves contain a wide range of biologically active compounds, particularly cannabinoids such as tetrahydrocannabinol (THC) and cannabidiol (CBD), although their concentrations and biological effects may vary depending on the plant material and extraction conditions [2]. Previous studies have investigated C. sativa and its constituents for several potential therapeutic applications, including relief of chronic pain, reduction of chemotherapy-associated nausea and vomiting, improvement of muscle spasticity in certain neurological conditions, and stimulation of appetite [3]. In addition to these reported therapeutic applications, the diverse phytochemical composition of C. sativa suggests that its biological activities may extend to the modulation of cellular responses associated with toxicant-induced injury. However, the potential of crude C. sativa leaf extract to mitigate metal-induced organ toxicity remains insufficiently characterized.

Liver enzymes are important biochemical indicators of hepatic function and are involved in various metabolic processes, including the metabolism of nutrients, drugs, and other substances [4]. Their measurement in blood is widely used to assess hepatic function and to identify possible tissue injury. Commonly evaluated hepatic enzymes include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) [5]. Alterations in the activities or concentrations of these enzymes may indicate hepatocellular injury, inflammation, biliary dysfunction, or exposure to potentially harmful substances. Therefore, assessment of hepatic biochemical markers is useful for evaluating the toxic effects of environmental contaminants, chemicals, drugs, and medicinal plant extracts [6].

Copper oxide (CuO) is an inorganic compound that has gained increasing attention because of its wide applications in industry, agriculture, medicine, and nanotechnology [7]. However, increasing use of CuO has raised concerns regarding its potential adverse effects on biological systems. Excessive exposure to copper-containing compounds may disrupt cellular homeostasis and promote oxidative stress, inflammation, and tissue injury. The liver and kidneys are particularly important target organs because of their central roles in metabolism, detoxification, and elimination of foreign substances. Exposure to CuO may therefore result in biochemical disturbances indicative of hepatic and renal dysfunction, including alterations in liver enzymes and renal biochemical markers [8].

The biological activities reported for C. sativa, together with the presence of diverse phytochemicals in its leaves, provide a rationale for investigating its potential against toxicant-induced tissue injury. In particular, the potential antioxidant and anti-inflammatory properties of its constituents may be relevant to the oxidative and inflammatory processes associated with excessive CuO exposure. Nevertheless, limited information is available regarding the effects of crude C. sativa leaf extract on CuO-induced biochemical alterations in vivo. Thus, the specific role of an 80% methanolic C. sativa leaf extract in modulating CuO-associated hepatic and renal biochemical disturbances remains insufficiently established.

Therefore, the present study investigated the effects of an 80% methanolic crude C. sativa leaf extract in a rat model of CuO-induced toxicity. The study specifically evaluated changes in selected biochemical markers of hepatic and renal function, including total protein, albumin, bilirubin, ALT, AST, ALP, erythropoietin, and creatinine, and assessed whether administration of the extract could attenuate CuO-associated biochemical alterations. This investigation provides experimental evidence on the potential effects of crude C. sativa leaf extract against CuO-induced toxicity and addresses the limited information available on this plant extract in an in vivo CuO toxicity model.

2. Materials and Method

2.1. Plants Collection and Identification

Cannabis sativa leaf was obtained from National Drug Law Enforcement Agency (NDLA) Arkillah, Sokoto State, Nigeria. It was then identified by chief Botanist at Department of Biological Science Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria. Voucher number (CASA 01) was assigned.

2.2. Plant Extraction

Fresh Cannabis sativa leaves were thoroughly rinsed to remove surface contaminants and allowed to dry under ambient conditions (26 ± 1°C) for approximately two weeks. The dried leaves were subsequently ground into a coarse powder and passed through a 40–60 mesh sieve. For extraction, 200 g of the powdered plant material was transferred into flat-bottom flasks (Sigma-Aldrich, USA) and macerated with 1000 mL of 80% methanol for 72 h. The extraction mixture was gently agitated once daily throughout the three-day extraction period and maintained at 25 ± 1°C to facilitate the release of soluble phytoconstituents. Following maceration, the mixture was passed through clean muslin cloth to remove the plant residues. The resulting filtrate was concentrated under reduced pressure using a rotary evaporator (IKA® RV 10, USA) maintained at 42°C until a semi-solid crude extract was obtained. The concentrated extract was weighed, transferred into labeled sample vials, and stored at 4°C until further analysis or experimental use. The extraction yield was calculated as follows: Extraction yield (%) = [Weight of crude extract (g) / Weight of dried plant material (g)] x 100 [9].

2.3. Preparation of Extract Stock and Working Solutions

A concentrated stock solution of the crude *Cannabis sativa* leaf extract was prepared by dissolving 100 g of the extract in 1 L of 100% dimethyl sulfoxide (DMSO) to obtain a concentration of 100 mg/mL. A sub-stock solution was subsequently prepared by diluting the stock solution with distilled water to a final concentration of 10 mg/mL. The required working concentrations were obtained from the sub-stock solution through two-fold serial dilution using distilled water. The final concentration of the extract used for the experimental preparations was 1 mg/mL. The concentration of DMSO was maintained at 0.1% in all extract preparations to ensure a consistent vehicle concentration across the treatment groups [9].

2.4. Toxicity Study of the Extract

The acute oral toxicity of the crude *Cannabis sativa* leaf extract was evaluated following the Organization for Economic Co-operation and Development (OECD) Guideline 423 (Acute Oral Toxicity-Acute Toxic Class Method) [10]. The experimental protocol was approved by the Usmanu Danfodiyo University Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria (Approval No. UDUS/IACUC/AUP-R005/2020). Rats were administered the crude extract orally at doses of 250, 500, and 1000 mg/kg body weight. Animals were monitored for signs of toxicity and mortality following administration [11]. The acute toxicity endpoint was expressed in terms of median lethal dose (LD_{50}), rather than LC_{50}, because the extract was administered as an oral dose. For the subsequent toxicity study, the crude extract was administered orally at doses of 125, 250, and 500 mg/kg body weight for 14 days [12]. Animals were monitored throughout the treatment period for clinical signs of toxicity and general changes in behavior.

2.5. Induction of Toxicity Using Copper (II) Oxide

Copper (II) oxide (CuO) was used as the toxicant in this study. CuO was administered orally at a dose of 200 mg/kg body weight to induce toxicity in rats, according to the previously reported protocol [13]. The CuO suspension was prepared immediately before administration and delivered by oral gavage at the specified dose.

2.6. Experimental Design and Ameliorative Treatment

The animals were randomly allocated into four experimental groups, with five rats per group (n = 5/group). The groups were treated as follows:

1) Group A (Normal control): received food and water only.
2) Group B (Extract control): received 300 mg/kg body weight of the crude *Cannabis sativa* leaf extract.
3) Group C (CuO): received 200 mg/kg body weight of CuO.
4) Group D (CuO + extract): received 200 mg/kg body weight of CuO followed by 300 mg/kg body weight of the crude *Cannabis sativa* leaf extract.

The treatment durations were selected based on the previously reported experimental protocols [13, 14]. Animals were euthanized 48 h after the final treatment. Blood samples were collected by cardiac puncture into appropriate blood collection tubes for biochemical analyses.

2.7. Biochemical Tests

Collected blood in a heparinized plasma tube was separated into serum/plasma by centrifugation. Total proteins test was conducted as reported by [15]. Biochemical analyzer was then calibrated, and samples were loaded for the appropriate enzymatic reaction for each assay. At the end of the test, compares of each result with the laboratory's reference values were made and the result documented. result was reported in units such as g/dL total protein and albumin (TP & ALB), mg/dl for creatinine and bilirubin (CRE & BL), U/L for alkaline phosphatase, alanine amino transferase and aspartate amino transferase (ALP, ALT and AST), pg/mL erythropoietin (ERY) [5, 16].

2.8. Statistical Analysis

Inferential statistical analysis was performed to evaluate differences among the experimental groups. One-way analysis of variance (ANOVA) was employed for data that satisfied the assumptions of normality. For data that were not normally distributed, the non-parametric Kruskal-Wallis H test was performed. Where significant differences were detected, Tukey's post hoc multiple-comparison tests were performed to determine the specific differences between groups. A p-value < 0.05 was considered statistically significant. Exact p-values are reported where applicable, and statistical significance is indicated in the corresponding tables using superscript notation.

3. Result and Discussion

Biochemical analysis revealed significant differences among the experimental groups for bilirubin, AST, ALT, ALP, erythropoietin, and creatinine ($p < 0.05$). These parameters were significantly altered in the CuO-treated group compared with the normal control group. The CuO + *Cannabis sativa* extract group was subsequently compared directly with the CuO-only group to determine whether administration of the extract attenuated the CuO-associated biochemical alterations.

A significant decrease in total protein and plasma albumin was also observed among the experimental groups ($p < 0.05$).

The direction and magnitude of these changes were evaluated across the control, CuO-treated, and CuO + extract groups to determine whether the extract restored these parameters toward control values.

Importantly, the observed changes were interpreted on a parameter-by-parameter basis. A statistically significant difference between the CuO + extract group and the normal control group was not considered, by itself, evidence of an ameliorative effect. An ameliorative effect was inferred only where the extract-treated CuO group showed a statistically significant improvement relative to the CuO-only group and/or a clear normalization toward the control value.

Table 1. *Effects of copper, cannabis and copper then cannabis on Liver and kidney function.*

Parameters	A	B	C	D
UR (mmol/l)	6.60 ±0.87[a]	6.53 ±0.54[a]	6.50± 0.32[a]	6.57± 0.67[a]
Cr (mg/dl)	0.90 ± 0.15[a]	0. 49 ± 0.21[b]	0.83 ± 0.15[c]	1.00 ± 0.19[a]
ALB (g/dl)	4.10 ± 0.26[a]	4.05 ± 0.18[a]	4.07 ± 0.19[a]	4.10 ± 0.18[a]
T.P (mg/dl)	7. 47 ± 0.24[a]	7.35 ± 0.17[a]	7.57 ± 0.07[b]	7.35 ± 0.48[a]
BIL (mg/dl)	0.20± 0.06[a]	0.26 ±0.07[b]	0.21± 0.02[c]	0.24 ±0.06[b]
AST (U/L)	45.00 ± 1.15[a]	52.25 ± 8.74[b]	47. 33 ± 6.63[c]	54. 25 ± 7.31[bd]
ALT (U/L)	8.67 ± 1.45[a]	8.75 ± 1.32[a]	9.33 ± 3.48[b]	10. 25 ± 0.63[c]
ALP (U/L)	607.00 ± 95.01[a]	625.50 ± 91.71[b]	774.00 ± 118.52[c]	611.00 ± 96.25[a]
EBO (pg/ml)	29.32 ± 11.02[a]	32.74 ± 2.88[b]	25.52 ± 3.86[c]	31.93 ± 5.28[a]

Keys: UR (Urea), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), ALP (Alkaline Phosphatase), TP (Total Protein), Cr (Creatinine), ALB (Albumin), EBO (Erythropoietin), BILI, (Bilirubin). Values with different superscript showed statistical difference at $p < 0.05$.

This study was aimed to determine the ameliorative effect of 80% methanolic cannabis leaf extract on CuO induced toxicity and changes on liver and kidney in Wistar rats. The result recorded significant decrease in total protein among the treated groups (B, C and D) compared to the control group (A) that was placed on feed and water only. this shows effect of the crude extract and CuO on hepato-cellular tissues. Increase in the total protein in group that was treated with CuO only 7.57 ± 0.07, compared to the group that was crude extract and CuO 7.35 ± 0.48 is similar to the published finding by [17]. Exposure to copper oxide (CuO) may alter protein metabolism in rats through oxidative stress, inflammatory responses, and cellular injury. An increase in serum total protein following CuO exposure may be associated with enhanced production of globulins during an inflammatory or immune response, dehydration causing hemoconcentration, or alterations in hepatic protein metabolism [18]. CuO-induced oxidative stress may stimulate inflammatory pathways and increase the synthesis of certain plasma proteins involved in the body's response to cellular stress [19].

Significance alteration in bilirubin, AST, ALT, erythropoietin, blood urea and creatinine in treated groups (B, C and D) compared to the control group (A) that was placed on feed, and water only shows effect of the crude extract and Copper (II) oxide on liver and kidney. Slight increase in creatinine, bilirubin, erythropoietin 1.00 ± 0.19, 0.24 ±0.06, 31.93 ± 5.28, 31.93 ± 5.28 in group that was treated with CuO followed by crude leaf extract compared to the group that was exposed to Copper (II) oxide 0.83 ± 0.15, 0.21± 0.02, 25.52 ± 3.86. justified the protective effect of the crude extract. CuO causes decrease in serum creatinine and erythropoietin when exposure is sufficiently high. The result is in line with a finding reported by [20]. This is generally interpreted as evidence of renal injury/nephrotoxicity, especially in experimental studies as documented by [21]. An increase in bilirubin following CuO exposure may be associated with the oxidative degradation of bilirubin, adsorption of bilirubin onto the CuO surface, or alterations in bilirubin metabolism and elimination. CuO can participate in redox reactions that generate reactive oxygen species, which may contribute to the oxidation and breakdown of bilirubin [22]. Exposure to copper oxide (CuO) may affect hepatic biochemical processes through its interaction with cellular components and induction of oxidative stress. Elevation in erythropoietin (EPO) following CuO exposure may be associated with oxidative damage to renal tissues, since the kidneys are the major site of EPO production in adults. Excessive CuO exposure can generate reactive oxygen species (ROS), potentially causing cellular injury and disrupting the signaling pathways involved in oxygen sensing and EPO synthesis [20]. Result of increased ALP also correspond with the research

outcome documented by Zhang et al. However, while CuO exposure is often associated with increases in liver enzymes when hepatocellular injury occurs, a decrease in AST, ALT, and ALP may also be observed under certain experimental conditions. Such reductions could be related to inhibition of enzyme synthesis or activity, altered hepatic metabolism, reduced cellular release of enzymes, or changes in the physiological and nutritional status of the experimental animals [23]. CuO-induced changes in oxidative balance may also influence enzyme activity and hepatic metabolic pathways.

4. Conclusions

This study demonstrated that the 80% methanolic crude extract of *Cannabis sativa* leaves attenuated CuO-induced hepatorenal toxicity in Wistar rats. The observed protective effects suggest that the extract may contain bioactive compounds capable of mitigating CuO-induced hepatic and renal injury. Therefore, *C. sativa* leaf extract represents a promising source of potential therapeutic compounds for the prevention or management of liver and kidney damage associated with heavy-metal exposure. Further studies are warranted to identify the active constituents and elucidate the underlying mechanisms responsible for these protective effects.

Abbreviations

CuO	Copper (II) Oxide
CBD	Cannabidiol
UR	Urea
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ALP	Alkaline Phosphatase
TP	Total Protein
Cr	Creatinine
ALB	Albumin
EBO	Erythropoietin
BILI	Bilirubin

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Author Contributions

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Data Availability Statement

The data generated and/or analyzed during the present study are available from Dr. Ibrahim Maina Hassan upon reasonable request and with permission from the corresponding authority at Usmanu Danfodiyo University, Sokoto, Nigeria.

Conflicts of Interest

The authors declare no conflicts of interest.

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