

Original Research Article

AMELIORATING EFFECT OF METHANOL EXTRACT OF FINGER ROOT BARK (*Uvaria chamae*) ON SOME SELECTED BIOCHEMICAL PARAMETERS OF CdCl₂ INDUCED TOXICITY IN WISTAR RATS

ABSTRACT

Background: High cadmium-induced injuries have necessitated the search for potent and cost-effective herbal management models. Hence, this study aimed at evaluating the ameliorating effect of methanol extract of finger root bark (*Uvaria chamae*) on some selected biochemical parameters of cadmium-induced toxicity in Wistar rats.

Methods: Thirty (30) rats weighing (84- 183) grams were obtained and kept in the animal house to acclimatize for eight (8) days prior to the experiment. The rats were grouped into five groups containing six rats each. Group 1 served as normal control; group 2 served as the negative control treated with 10mg/kg bw CdCl₂; group 3 contained rats fed with 500mg/kg bw plant extract; Group 4-5 contained cadmium-intoxicated rats treated with *Uvaria chamae* (250mg/kg body weight and 250mg/kg body weight) respectively for 13 days. Thereafter, the rats were sacrificed, and blood samples were collected through the ocular puncture for biochemical analysis. Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), as well as lipid profiles were assessed. Tissue antioxidant levels of reduced glutathione (GSH), malondialdehyde (MDA), and activities of antioxidant enzymes such as superoxide dismutase (SOD) were also determined.

Result: CdCl₂ caused a significant derangement in lipid profile, increasing serum triglyceride, total cholesterol and low-density lipoprotein (LDL) while high-density lipoprotein (HDL) level was diminished. Liver biomarkers (ALT, AST, ALP) were also significantly elevated in the serum relative to the control animals following exposure to CdCl₂. Activities of antioxidant enzymes in the serum were markedly inhibited by CdCl₂ exposure. Treatment with *Uvaria chamae* extract caused a dose-dependent restoration of all biochemical parameters determined.

Conclusion: The findings show the potential usefulness of *Uvaria chamae* extract in managing certain diseases.

Keywords: Biomarkers, Cadmium, Oxidative stress, Lipid, liver

ABBREVIATIONS

ALP: Alkaline phosphate

ALT: Alanine Amino Transferase

AST: Aspartate Amino Transferase

CdCl₂: Cadmium Chloride

GSH: Glutathione

H₂O₂: Hydrogen peroxide

HDL-Ch: High-Density Lipoprotein Cholesterol

LDL-Ch: Low-Density Lipoprotein Cholesterol

MDA: Malondialdehyde

OECD: Organization for Economic Cooperation and Development

ROS: Reactive oxygen species

SOD: Superoxide Dismutase

1.0 INTRODUCTION

Environmental pollution has remained an indispensable makeup of our general surroundings today.

According to Guo et al. [1], it is an unpleasing modification of our environment entirely due to man's direct

and indirect activities on organisms' energy patterns, radiation levels, and chemical and physical components. Since the late 19th and early 20th centuries, metals have had a tremendous rise in environmental pollution. The environmental constituents, including the biosphere, have been constantly threatened by extravagant contamination of heavy metals emitted from various industries. Many of these heavy metals have been reported to have adverse effects [2]. Cadmium (Cd) is a toxic, naturally occurring, non-essential transition metal. Cadmium appears as a pollutant derived from various commercial sources in the environment. Due to its high toxicity, it has remained a risk to the health of both plants and human beings. According to Hideaki *et al.* [3], studies have shown that excessive cadmium exposure interferes with DNA repair, induces genomic stability, and improves the generation of reactive oxygen species. The presence of cadmium in cells disrupts cellular activities, including cell differentiation, proliferation, and apoptosis. The most dangerous characteristic of cadmium is that it accumulates throughout a lifetime due to its long biological half-life. In animal models, some plants have proven to reduce plasma levels of TC, TG and LDL-C [4]. Various researchers have proposed medicinal plants to be an alternative remedy to eliminate the biochemical implications caused by cadmium toxicity. Finger Root (also known as *Uvaria chamae*) is a yellow fruit comprised of a non-poisonous pulp and known to be widely consumed. In Nigeria, *Uvaria chamae* is locally called Kaskaifi or Lukuki by the Hausa, Mmimi Ohea by the Igbos and Oko-oja by the Yoruba in Nigeria [5]. The roots of *Uvaria chamae* have been reported to treat various health issues, including inflammation of the Mucous membrane, bronchitis and gonorrhoea, dysentery, fever, and relieve childbirth pains [6]. etc. *Uvaria chamae* has been reported to have antibacterial, cytotoxicity [7], antimalarial, hepatoprotective and antiviral properties [7]. Using *Uvaria chamae* to remedy cadmium intoxicated Wistar rats has never been accounted for. Hence, this study investigates *Uvaria chamae* ameliorating potentials in some selected biochemical parameters in CdCl₂ induced toxicity in Wistar rats.

2.0 MATERIALS AND METHODS

2.1 COLLECTION OF PLANT SAMPLES

Finger roots (*Uvaria chamae*) were collected from the premises of the Umuebele community in Oyibo Local Government Area of Rivers State. The plant was identified by a botanist from the forestry department, Federal University of Technology Owerri PMB 1526, Owerri, Imo State, Nigeria, and the

voucher specimen was deposited in the department's herbarium with the voucher specimen Voucher No. IHF=22126.

2.2 PREPARATION OF PLANT SAMPLE EXTRACT

The preparation of the plant extract adopted the method described by Madubunyi [8]. The Finger roots (*Uvaria chamae*) were washed thoroughly and air-dried at room temperature ($20\pm 27^{\circ}\text{C}$). They were pulverized using a hand milling machine (TSK-948 West Point, France). The methanol extract of *Uvaria chamae* was prepared using 500g of the powdered leaves mixed in 1839ml of ethanol for 70hours with occasional storing. The mixture was filtered using a muslin cloth and then with Whatman filter paper No.1. The chaff was discarded, and the filtrate was evaporated and dried using a rotary evaporator (Cole-Parmer model SB-1200) at 60°C . The filtrate was stored in the refrigerator until it was ready for use.

2.2 EXPERIMENTAL ANIMAL

Thirty (30) male Wistar rats (84-120g) were purchased from an animal farm at the University of Nigeria Nsukka and used for this study. The animals were housed in standard aluminium cages (6 animals per cage) in clean conditions at an ambient temperature of 25°C with a 12-hour light /dark cycle. The rats were fed with water *ad libitum* and a commercialized poultry feed called " Chikun feed ". The animals were acclimatized for 8 days before the commencement of the experiment. The Principles of Laboratory Animal Care, NIH [9], were followed throughout this study. All experimental procedures were conducted according to the animal ethics committee.

2.3 PREPARATION OF CADMIUM CHLORIDE SOLUTION AND *UVARIA CHAMAE* ROOT EXTRACT

The method described by Al-Hashem et al. [10] was employed. Cadmium chloride (CdCl_2) was dissolved in sterile distilled water to ascertain a required concentration of which the rats received an equivalent of 1.0ml, which was administered orally with a cannula. *Uvaria chamae* extract was reconstituted in distilled water to achieve the desired doses of 250 and 500 mg/kg body weight used in the animal experiment.

2.4 EXPERIMENTAL DESIGN

The animals were allowed to acclimatize for 8days before the commencement of treatment. The animals were marked and divided into five groups (1-5) of six (6) rats per group.

Table 1: Grouping and Treatment of Animals

Groups	Treatment	Duration
Group 1	Feed + H ₂ O at 5ml/kg	13 days
Group 2	10mg/kg body weight of cadmium chloride (CdCl ₂)	13 days
Group 3	500mg/kg body weight of <i>Uvaria chamae</i> extract	13 days
Group 4	10mg/kg body weight of CdCl ₂ + 250mg/kg body weight of <i>Uvaria chamae</i> extract	13 days
Group 5	10mg/kg body weight of CdCl ₂ + 500mg/kg body weight of <i>Uvaria chamae</i> extract	13 days

2.5 ACUTE ORAL TOXICITY

The acute oral toxicity of the plant extract was carried out strictly in adherence to the Organization for Economic Cooperation and Development (OECD) guidelines. Four animals per dose were used for the experiment. Overnight fasted rats were orally fed with the methanol extract of *Uvaria chamae* in dose levels of 200, 800, and 1000 mg/kg body weight, respectively. The animals were consciously observed for their behavioural, touch response, pain response and spontaneous activity, and autonomic (defecation and urination) profiles for 24 h. After 24 h, the animals were observed for 14 days for mortality.

2.6 COLLECTION AND PREPARATION OF SAMPLES

At the end of the administration period, the animals were anaesthetized on the 14th day, and blood samples were collected via ocular puncture. The blood samples were stored in clean vacutainer tubes and centrifuged at 4000 g for 15 minutes. The serum was used to estimate biochemical markers using Randox Diagnostic kits.

2.7 DETERMINATION OF BIOCHEMICAL PARAMETERS

The plasma samples were used for the determination of alanine transaminase (ALT), aspartate transaminase (AST) [11], as the liver function indicator, Malondialdehyde (MDA) [12], as the lipid peroxidation indicator, Superoxide Dismutase (SOD) [13], and Glutathione (GSH) as the antioxidant

enzymes. The Total plasma Cholesterol (T-Ch) [14], Triglycerides (TG) [15], High-Density Lipoprotein Cholesterol (HDL-Ch) [16].

2.8 STATISTICAL ANALYSIS

Data obtained was expressed as mean \pm SD and statistically analyzed using one-way analysis of variance (ANOVA) with Turkey's multiple comparison post hoc tests to compare the level of significance between the test groups. The values of $p < 0.05$ were considered significant.

3.0 RESULT

3.1 Toxicity Study of *Uvaria chamae* extract

Acute toxicity studies showed no mortality up to 1000 mg/kg given as a single oral administration mg/kg. The study was done at three different dose levels (250 and 500 mg/kg).

3.2 Result of ethanol extract of *Uvaria chamae* root bark on alanine aminotransferase (ALT) activity of cadmium-intoxicated rats

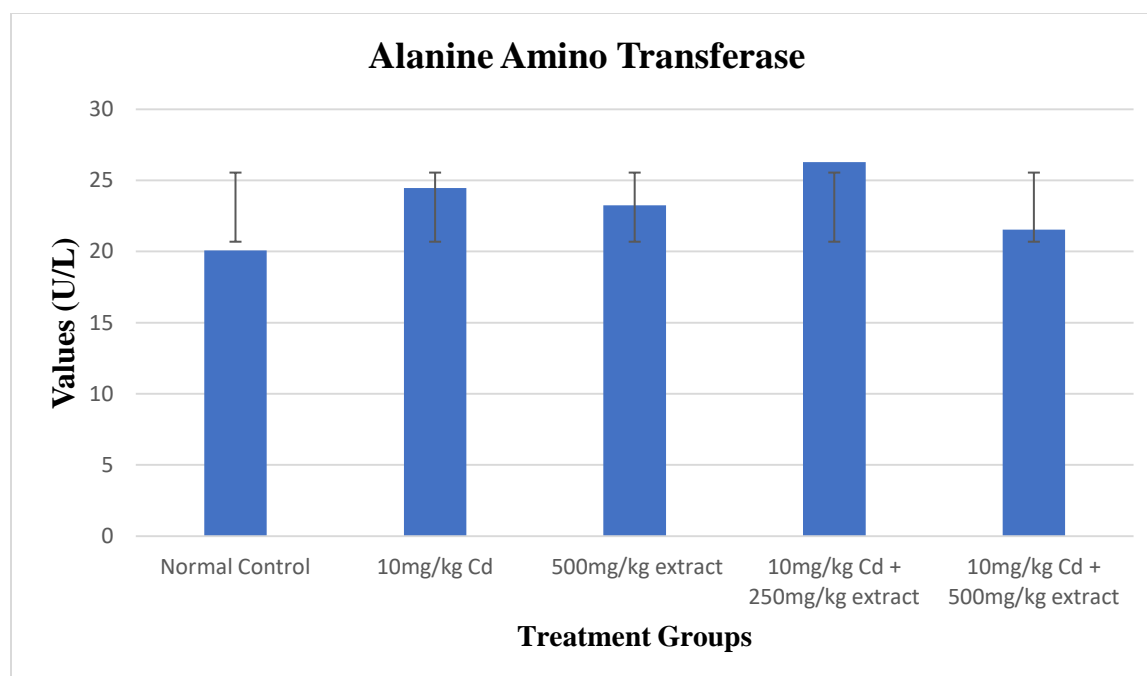


Fig. 1. Effect of *Uvaria chamae* Extract on Alanine Amino Transferase (ALT) serum enzymes

Values are expressed as mean±SD (n=6). * $p<0.05$ when compared with the normal control.

Data for the ALT enzyme activities were significantly ($p<0.05$) increased in the untreated cadmium-intoxicated group (24.47 ± 0.66) compared to the normal control (20.08 ± 0.45). At 500mg/kg of the cadmium-intoxicated group treated group, the plant extract significantly decreased ALT compared to the normal control.

3.3 Result of ethanol extract of *Uvaria chamae* root bark on Aspartate Amino Transferase (AST) activity of cadmium-intoxicated rats

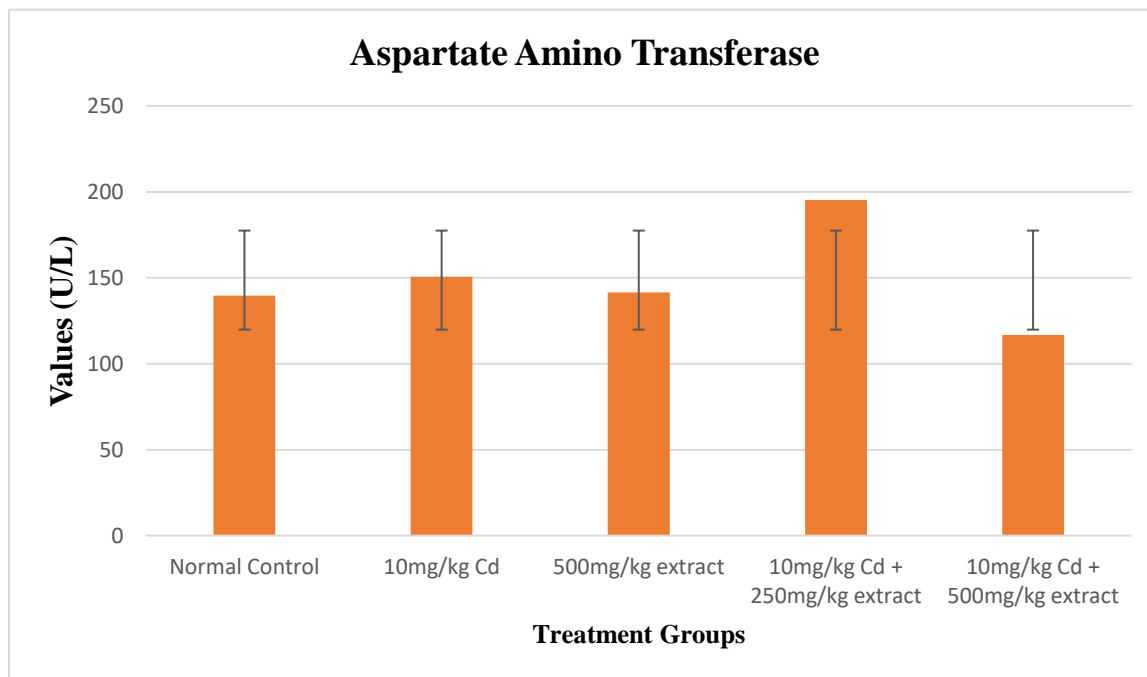


Fig. 2. Effect of *Uvaria chamae* Extract on Aspartate Amino Transferase (AST) serum enzymes

Values are expressed as mean \pm SD (n=6). * $p < 0.05$ when compared with the normal control.

The aspartate amino transferase (AST) activity of rats administered 10 mg/kg Cd + 250 mg/kg extract showed a significantly ($P < 0.05$) elevation compared to the normal control. There was no significant difference in the other groups extract 500mg/kg, cadmium 10mg/kg and Cadmium, 10 mg/kg + Extract, 500 mg/kg when compared to the control group.

3.4 Result of ethanol extract of *Uvaria chamae* root bark on Alkaline phosphate (ALP) of cadmium-intoxicated rats

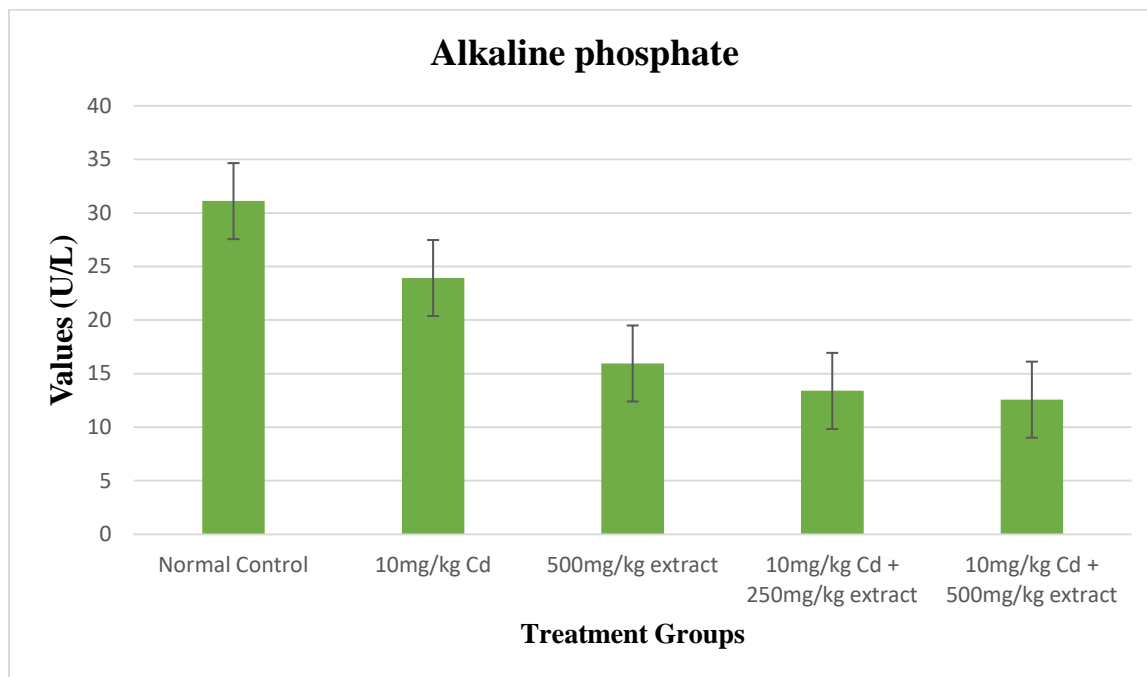


Fig. 3. Effect of *Uvaria chamae* Extract on Alkaline phosphate (ALP) serum enzymes

Values are expressed as mean \pm SD (n=6). * $p < 0.05$ when compared with the normal control.

The alkaline phosphate (ALP) activity of rats administered from (Group 2-5) was significantly ($P > 0.05$) lower compared to the normal control.

3.5 Result of the ethanol extract of *Uvaria chamae* root bark on malondialdehyde (MDA) concentration of cadmium-intoxicated rats.

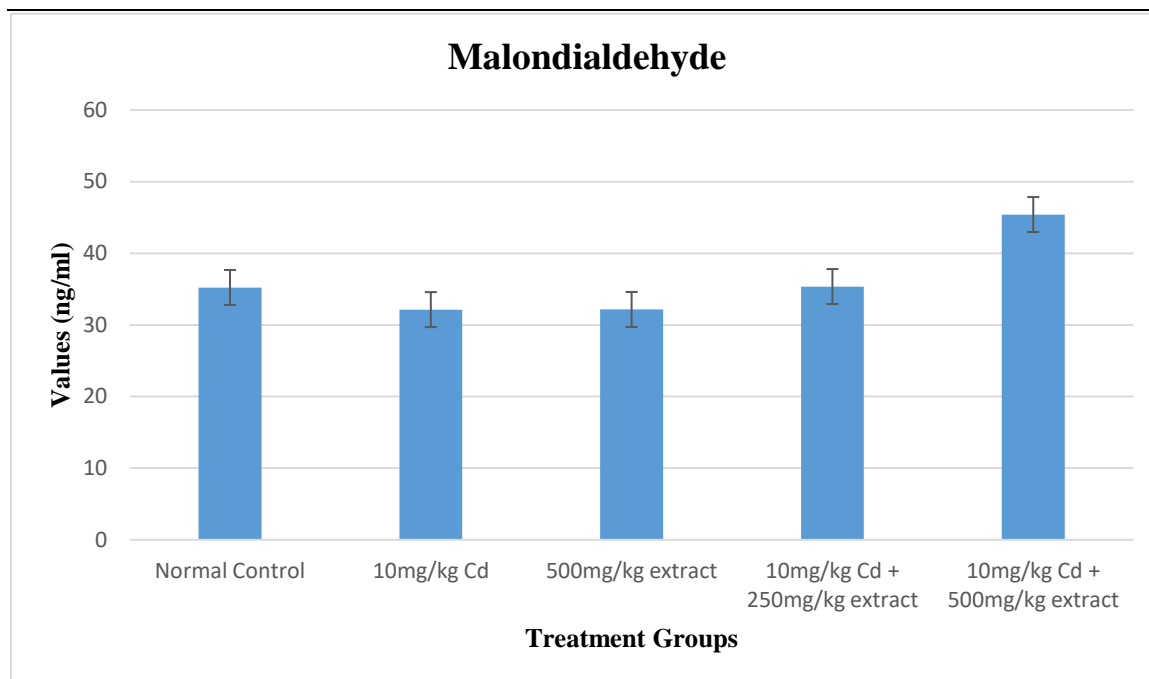


Fig. 4. Effect of *Uvaria chamae* extract on malondialdehyde (MDA) concentration of cadmium-intoxicated rats.

Values are expressed as mean \pm SD (n=6). * $p<0.05$ when compared with the normal control.

Data for the MDA enzyme activities were significantly ($p<0.05$) reduced in the cadmium-intoxicated group compared to the normal control. Supplementation of methanol extract of *Uvaria chamae* at 250 mg/kg and 500 mg/kg significantly elevated MDA activity compared to the control animals.

3.6 Result of the ethanol extract of *Uvaria chamae* root bark on superoxide dismutase (SOD) activity of cadmium-intoxicated rats

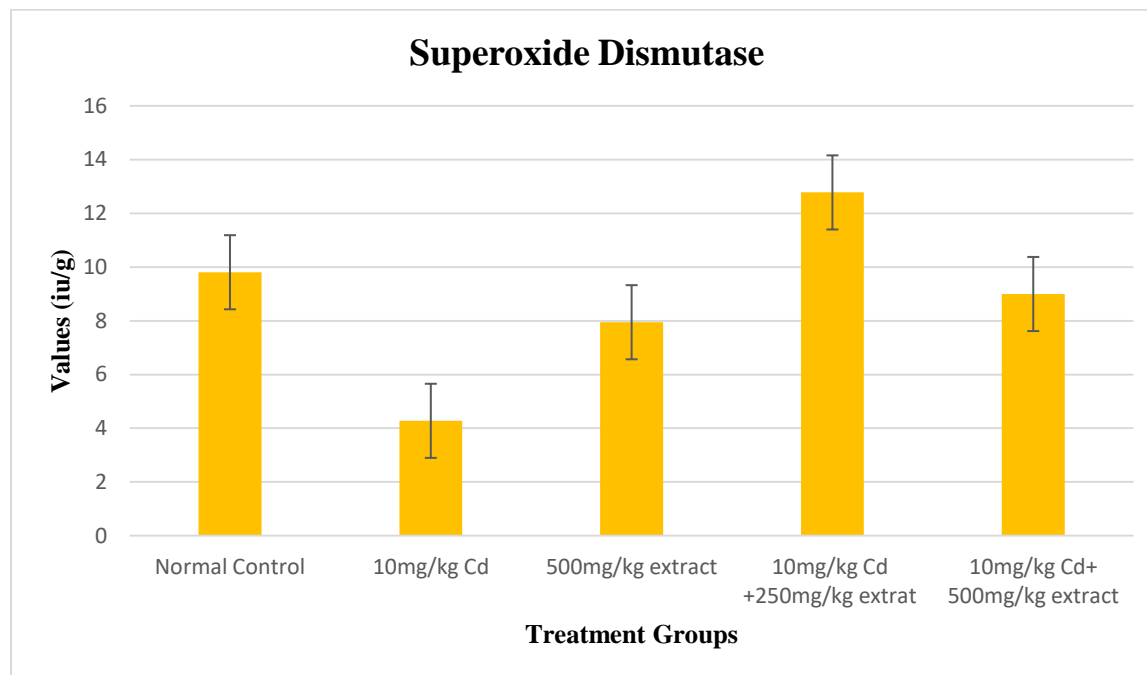


Fig. 5. Effect of *Uvaria chamae* Extract on Superoxide Dismutase (SOD) concentration of cadmium-intoxicated rats.

Values are expressed as mean \pm SD (n=6). * $p<0.05$ when compared with the normal control.

Data for the SOD enzyme activities were significantly ($p<0.05$) reduced in the cadmium-intoxicated group (4.28 ± 0.65) compared to the normal control (9.81 ± 1.10). However, supplementation of methanol extract of *Uvaria chamae* at 250 mg/kg and 500 mg/kg significantly elevated SOD activity compared to the control animals.

3.7 Result of the ethanol extract of *Uvaria chamae* root bark on Glutathione (GSH) Concentration of cadmium-intoxicated rats.

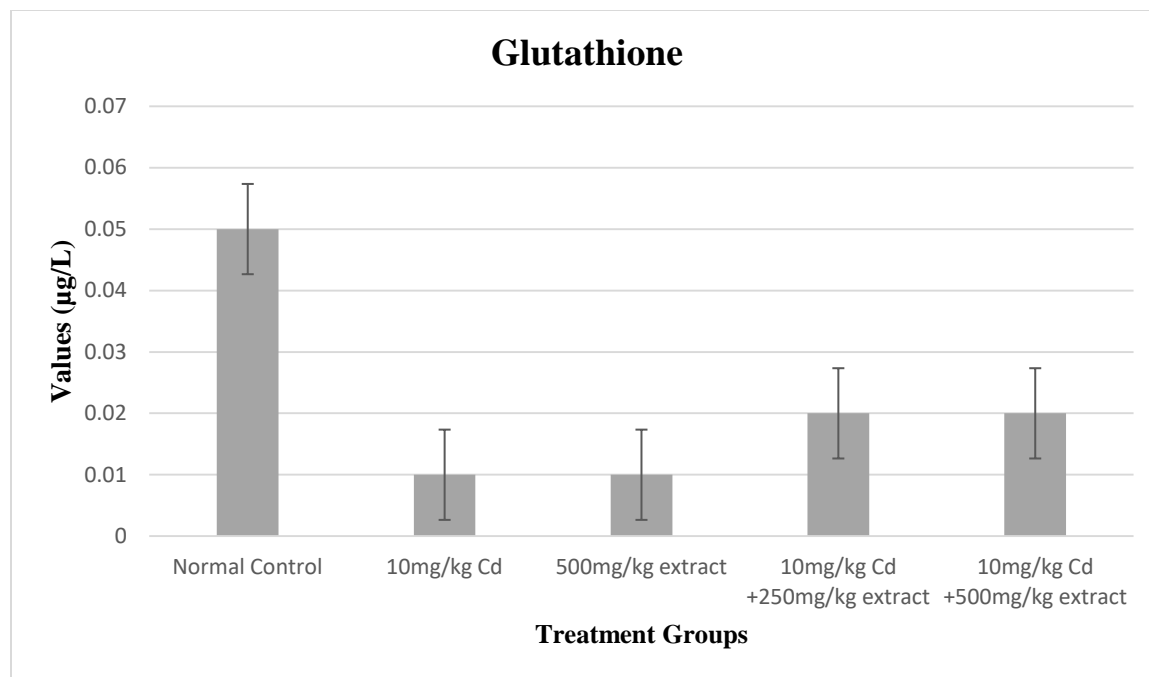


Fig. 6. Effect of *Uvaria chamae* Extract on Glutathione (GSH) Concentration of cadmium-intoxicated rats.

Values are expressed as mean \pm SD (n=6). * $p<0.05$ when compared with the normal control.

Data for the GSH enzyme activities were significantly ($p<0.05$) reduced in the cadmium-intoxicated group compared to the normal control. Supplementation of methanol extract of *Uvaria chamae* at 250 mg/kg and 500 mg/kg significantly elevated GSH activity compared to the control animals.

3.8 Result of the ethanol extract of *Uvaria chamae* root plant on the lipid profile parameter of cadmium-intoxicated Wistar albino rat

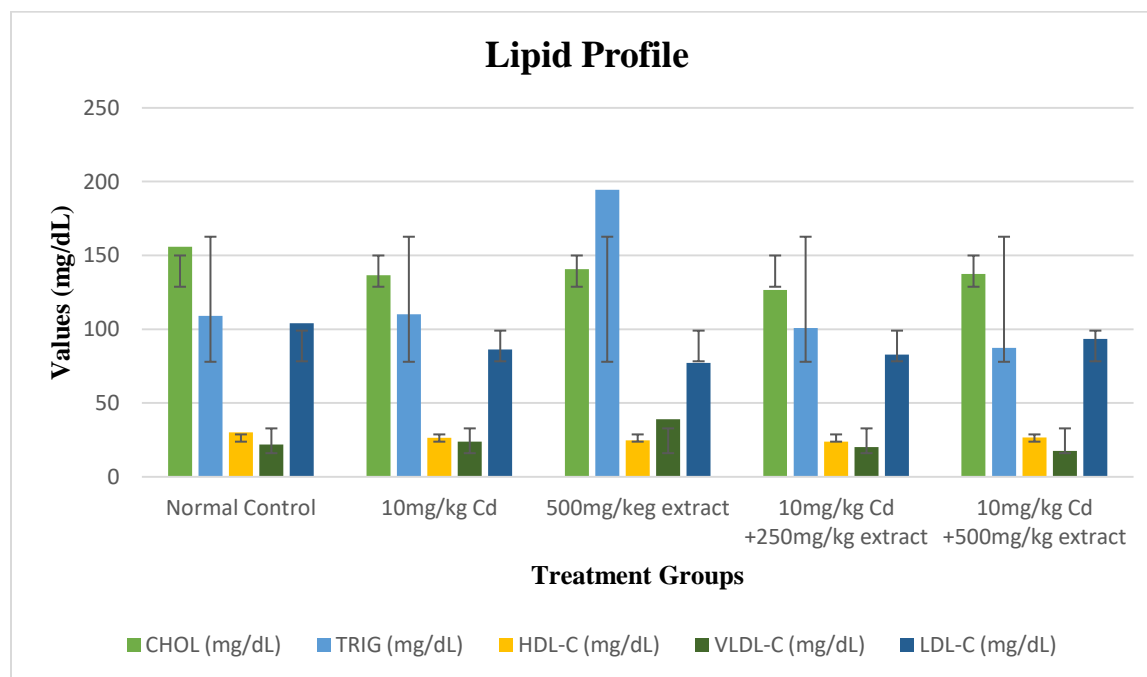


Fig. 7. Effect of *Uvaria chamae* extract on the lipid profile parameter of cadmium-intoxicated wistar albino rat

Values are expressed as mean \pm SD (n=6). * $p < 0.05$ when compared with the normal control.

Exposure to CdCl_2 resulted in marked ($p < 0.05$) derangement in lipid profile in all tissues of experimental animals analyzed. Supplementation with *Uvaria chamae* extract caused a dose-dependent restoration of deranged lipid profile comparable to the normal control group.

4.0 DISCUSSION

This study evaluated the ameliorating effect of methanol extract of finger root bark (*Uvaria chamae*) on selected biochemical parameters of CdCl₂ induced toxicity in Wistar rats.

From our study, the exposure of experimental rats to CdCl₂ toxicity resulted in a significant elevation in the serum level of these biomarkers compared to the normal control. The rise in the serum levels of these biomarkers indicates free-radical induced oxidative damage to the hepatocytes, as shown by the serum levels of these biomarkers. A rise in the ALT level usually accompanies a rise in AST activity. ALP is an abundant enzyme in hepatocyte membranes and is used to screen for cholestasis or biliary obstructions [17]. The rise of these serum biomarkers during liver diseases is due to their occupancy in the hepatocyte. If the liver cells are damaged, they only leak into the bloodstream, and the spill-over of the enzyme into the blood is a good indicator of abnormal cell damage and a sign of severe liver disease [18]. ALT, AST and ALP were on the increase due to the exposure to cadmium-induced oxidative attack on the liver cells. However, treatment with *Uvaria chamae* extract showed significant dose-dependent (250mg/kg and 500 mg/kg plant extract) restoration of these biomarkers (ALT, AST and ALP) activities in the serum and tissue homogenates to the negative control. The result of the work was in agreement with the previous work of Madubunyi [8] and suggested the hepatoprotective potentials of *Uvaria chamae* extract, which can be utilized in the management of liver diseases.

Lipid peroxidation is an established mechanism of cellular injury and indicates oxidative stress in cells and tissues [19]. It is known that the quantity of MDA is an intensity index of the peroxidation process of poly-unsaturated fatty acids (PUFAs) contained in food [20]. There was a significant dose-dependent (250mg/kg and 500 mg/kg plant extract) increase in MDA concentration in the treated group relevant to the untreated group. These results suggest that the ethanol extract of *Uvaria chamae* can reverse lipid peroxidation. The outcome of this work tallied with the previous findings made by Miaffo et al. [21], who reported a decrease in MDA level in rats administered with aqueous extract of the plant species *Combretum molle* twigs.

The primary defense against the superoxide anion is Superoxide dismutase (SOD), which converts the superoxide anion into hydrogen peroxide and water. Some studies suggest that overexpression of SOD is harmful to cells [22]. Reactive oxygen species (ROS) present in many cells with overexpressed SOD

have been attributed to elevated levels of hydrogen peroxide (H_2O_2) and accompanying oxidative damage resulting from hydroxyl radical formation [23]. In the present study, administration of $CdCl_2$ -exposed animals with *Uvaria chamae* extract reactivated the activity of SOD in the serum, and this is in agreement with Nwobodo et al. [24].

Glutathione is a tri-peptide comprised of the amino acid L-cysteine, L-glutamic and glycine. Glutathione functions as a water-soluble antioxidant at the cellular level and is directly involved in specific detoxification reactions that protect the body against dangerous substances. Several shreds of evidence are present that Cd^{2+} is associated with oxidative stress because this metal can alter the antioxidant defense system in various tissues of animals, causing a depletion in the level of GSH and alteration in the activity of antioxidant enzymes and a change in the structure of cell membrane through a process of lipid peroxidation, this was stated by Cuypers et al. [25]. Cadmium may directly induce oxidative stress causing lipid peroxidation, resulting in protein modification and membrane gradient alteration, resulting in loss of integrity and irreversible damage [26]. A significant reduction in the level of GSH in the liver cells increases the hepatotoxicity caused by cadmium, which is in line with the research finding of Satarug et al. [27].

The result of this study revealed a significant ($p > 0.05$) decrease in the level of the enzyme parameter (GSH). Treatment with *Uvaria chamae* extract to the $CdCl_2$ -exposed animals resulted in a significant dose-dependent elevation (250mg/ kg and 500 mg/kg plant extract) compared to the normal control. The increase in the level of GSH in the treatment group indicates that the plant extract helped boost the GSH concentration in the cytosol, inducing oxidative stress. This result is in line with previous findings made by Asagba et al. [28], who reported that *Hibiscus sabdarbifolia anthocyanins* are potent antioxidants and consonant with the reported protective effect of antioxidant nutrients against Cadmium-induced oxidative stress and lipid peroxidation in the liver and kidney.

Information on the susceptibility of the heart to atherosclerosis and its associated coronary heart disease could be provided by the cholesterol profile. [29]. The reduction in HDL-C at all doses investigated may not be clinically beneficial to the animals since the rate at which high plasma cholesterol is carried to the liver was also decreased. Furthermore, the enhanced level of cholesterol and LDL-C may suggest

cardiovascular risk in the animals. This is supported in the present study by increasing the computed atherogenic index, a helpful indicator of cardiovascular diseases [30].

CONCLUSION

From the results obtained from our study, we can conclude that the best dose of *Uvaria chamae* extracts that yield the best result with good efficacy and less hepatotoxicity on biomarker enzymes is 500 mg/kg. It could be a potential source of natural antioxidants that could be used as a therapeutic agent to prevent or treat degenerative diseases associated with oxidative stress. However, it is recommended that further studies be carried out to ascertain the therapeutical potency of this plant when used by humans.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study

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