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PROTECTIVE ROLE OF METHANOL EXTRACT OF *Carpolobia lutea* ROOT AGAINST CADMIUM-INDUCED CHANGES IN BIOCHEMICAL AND ANTIOXIDANT INDICES IN LIVER OF MALE WISTAR RATS

Akinola, A.O.[1,*], Oyeyemi, W.A.[2], Omotoso, D.R.[3], Daramola, O.O.[2] and Raji, Y.[4]

[1]Department of Physiology, University of Medical Sciences, Ondo-City, Ondo State, Nigeria
[2]Department of Physiology, Igbinedion University, Okada, Edo State, Nigeria
[3]Department of Anatomy, Redeemer's University, Ede, Osun State, Nigeria
[4]Laboratory for Reproduction and Developmental Programming, Department of Physiology, University of Ibadan, Ibadan, Nigeria
* Corresponding Author's Email: aakinola@unimed.edu.ng
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ABSTRACT

Oxidative stress and impaired antioxidant system have been implicated in the pathophysiology of various disease conditions associated with Cadmium (Cd) toxicity. *Carpolobia lutea* (*C. lutea*), has been shown to possess antioxidant properties. *Carpolobia lutea* root was obtained in Ijare via Akure, authenticated at Forestry Research Institute of Nigeria. Methanolic extract of *Carpolobia lutea* (MCL) was obtained by Soxhlet extraction and subjected to Gas Chromatography-Mass Spectrometry (GC-MS) to identify chemical compounds in the extract. Thirty male Wistar rats (150-170 g) were used in this study and treated as follows: Control (1 mL/kg body weight (bw) distilled water), Cd (2 mg/kg bw), Cd+MCL (2 mg/kg+100 mg/kg bw), Cd+MCL (2 mg/kg+200 mg/kg bw), MCL (100 mg/kg bw), MCL (200 mg/kg bw). The administration of *C. lutea* was done orally for eight weeks, and a single dose of 2 mg/kg Cd was administered intraperitoneally. Liver levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and histology were assessed. The GC-MS result showed the presence of 21 compounds. The *C. lutea* extract significantly reduced ($P<0.05$) ALP, AST and MDA while a significant increase was observed in SOD and CAT activities which were initially altered by administration of cadmium. The *C. lutea* extract also significantly attenuated the histopathological alterations of the liver rats. *C. lutea* root extract attenuated cadmium-induced toxicity which are indications of its antioxidant potential and may be responsible for its protecting effect against coadmium-induced liver damage.

Keywords: *Carpolobia lutea,* Cadmium, Superoxide dismutase, Liver toxicity, Rats

INTRODUCTION

Carpolobia lutea, a medicinal plant which possesses various active constituents with therapeutic properties that may offer an exciting opportunity for novel drug development. It belongs to the family of Polygalaceae and commonly known as cattle stick (English), Ikpafum (Ibibio, South-ern Nigeria), Agba or Angalagala (Igbo-Eastern Nigeria) and Oshunshun (Yoruba-Western Nigeria) (Etukudo, 2003). It is a shrub with an average height of 5 m and occurs as a dense overgrowth (Akpan *et al.*, 2012).

The phytochemical screening of different parts of *C. lutea* revealed the presence of tannins, saponins, flavonoids, cardiac glycosides, and anthraquinones (Nwidu *et al.*, 2015). Traditionally, various parts of the *C. lutea* have been claimed to be used in the management of several ailments. Apart from its aphrodisiac property, it is generally used for headaches, general pain and to prevent sleep due to fatigue (Mitaine-Offer *et al.*, 2002). It also has analgesic and androgenic properties. It is reputed to cure rheumatism, insanity, fever, dermal infection, vermifuge, venereal diseases, and to combat sterility and promote childbirth (Muanya and Odukoya, 2008). Pharmacologically, the *C. lutea* leaf has been reported to have antiulcer and antidiarrheal activity in rodents (Nwafor and Bassey, 2007), gastro-protective effect in rodents (Nwidu and Nwafor, 2009), anti-nociceptive effect in mice (Nwidu *et al.,* 2011), anti-inflammatory, neuro-pharmacological activity in rodents (Nwidu, 2010) and enhanced sexual activity in male rabbits (Dare *et al.,* 2015).

Cadmium (Cd) is a ubiquitous environmental pollutant, characterised by its toxicity in various organs (Gunnarsson *et al.,* 2003). The significant sources of Cd exposure are contaminated underground water, seafood, occupational activities (mining, batteries production) as well as

industrial activities (smelting, refining of metals), incineration of municipal waste (Siu *et al*., 2009) and tobacco smoke (Blanco *et al*., 2007). Cadmium exposure can result in a series of adverse effects, such as hepatic dysfunction, osteomalacia, pulmonary oedema, renal and testicular damage due to its accumulation in various organs, particularly the liver, testes and kidney, in both humans and animals (Arroyo *et al*., 2012). Liver as well as the kidney are organs that are important for metabolism, detoxification, storage and excretion of xenobiotics and their metabolites, and are also susceptible to damage (Brzoska *et al*., 2003).

Cadmium is a hazardous pollutant found in the environment, some occupational practices and in industrial effluent. A lot of experiments using animals have shown that the toxic effect of cadmium is manifested through generation of reactive oxygen species (ROS) and its attendant interference with antioxidant system of cells. *C. lutea* root extract has been reported to contain phytochemical compounds which include alkaloids, tannins, saponins, anthraquinones, flavonoids, cardiac glycosides, terpenes and simple sugar, which have been shown to possess antioxidant properties (Akinola *et al*., 2020).

Therefore, the present work evaluates the effects of methanol extract of *C. lutea* root on biochemical and redox status in Male Wistar rats' liver treated with Cadmium.

MATERIALS AND METHODS

Plant collection and extraction

Carpolobia lutea G. Don root was obtained from Ijare via Akure, Ondo state. The plant was authenticated at the herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Voucher number 109784 was assigned to the specimen.

Air dried root of *C. lutea* was pulverised, and 5.20 kg of the pulverised root was subjected to Soxhlet extraction using pure methanol. The extraction was concentrated with a vacuum-distilled rotary evaporator. The percentage yield for the extract was 1.7%. A fresh solution of the methanol extract of *C. lutea* root was prepared in distilled water (vehicle).

In-vitro antioxidant activity of *Carpolobia lutea*

Determination of total phenol content

The total phenol content in the methanol extract of *C. lutea* root was determined by the method described by Gulcin *et al.* (2003) using the Folin ciocalteu's phenol reagent which is an oxidising reagent. The total phenol content in the methanol extract of *C. lutea* root was compared with garlic acid and expressed as mg GAE/g.

Determination of total antioxidant capacity

Total antioxidant capacity (TAC) of the methanol extract of *C. lutea* root was estimated as reported by Prieto *et al.* (1999). The method is based on the reduction of Molybdenum (VI) to Molybdenum (V) by the extract and the subsequent formation of a green phosphate/Molybdenum (V) complex at an acidic pH. The result was recorded in relative to the standard (ascorbic acid) and expressed as mg AAE/g.

1,1-diphenyl-1-picrylhydrazyl hydrate radical scavenging activity

The 1,1-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical scavenging ability of the methanol extract of *C. lutea* root was determined using the stable radical DPPH as described by Brand-Williams *et al.* (1995).

Metal chelating activity

The metal chelating activity of methanol extract of *C. lutea* root was carried out using ferrous ion-chelating (FIC) method as reported by Singh and Rajini (2004).

Identification of chemical compounds in *C. lutea* using gas chromatography-mass spectrometry

The volatile solution of methanol extract of *C. lutea* root was partitioned and analysed using a gas chromatograph mass spectrometer (GC-MS). The GC-MS instrument equipped with GC: Aligent 7890 A, MS: MS detector 5975C, Ionization for MS: Electron Impact Ionization, Mass Analyzer: Quadrupole, Software: Data Analysis, Library: (Scarfone *et al*., 2008), column: HP 5 ms, Dimensions: 30 mm X 0.25 mm ID x 0.25 μm film thickness, initial temperature is 0 to 40°C 2 min hold time, ram temperature is 10°C to 310°C 10 min is the hold time, total time is 34 min, carrier gas is helium, flow (ml/min) is 1.0, split flow: 1

ml/min, injection volume: 1 µl, Scan mass range: 30 m/z-600 m/z and polarity +ve. The spectrum of the unknown compound was compared with the spectrum of the known compounds in the library.

Experimental animals

Thirty male Wistar rats with an average weight of 120 gram housed in well-ventilated cages in the Central Animal House, University of Medical Sciences, Ondo City, Ondo State were used for this study. They were kept under standard laboratory conditions, fed with standard rat pellets (Ladokun feeds Limited, Ibadan, Nigeria) and allowed free access to water. They were acclimatised for two weeks. All the procedures used in this study followed the guiding principles for research involving experimental animals as recommended by the Declaration of Helsinki as well as the Guiding principles in the use and care of animals (World Medical Association, American Physiological Society, 2002).

Animals grouping and treatment

Animals were randomly grouped into six categories, with each group consisting of five animals and treated as follows: Control, Cd (2 mg/kg), Cd+Methanol extract of *Carpolobia lutea* (MCL), (2 mg/kg+100 mg/kg), Cd+MCL (2 mg/kg+200 mg/kg), MCL (100 mg/kg), and MCL (200 mg/kg).

The animals were treated with MCL root orally for eight weeks, and a single dose of Cd was given intraperitoneally on the first day of administration. The animals were anaesthetised with 50 mg/kg of sodium thiopentone a day after the last administration of the extract. They were dissected to harvest the liver used for biochemical, redox assays and histology.

Biochemical and redox assay

The harvested liver (2.5 g) was washed in 10 mL of ice-cold 1.15% KCl solution, blotted with filter paper, and homogenised in 5 mL of ice-cold phosphate buffer saline (PBS) using Teflon homogeniser fitted into a microfuge tube chilled in ice. Each sample was centrifuged at 3,000 × g for 15 min. The supernatant was obtained and used for the assays of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) as well as superoxide dismutase (SOD), catalase and malondialdehyde (MDA) activities.

Liver biochemical assay

Alkaline phosphatase, ALT and AST activities were assayed using Randox commercial enzyme kit. The assays were carried out as described in the kit manual procedures according to Reitman and Frankel (1957).

Liver antioxidant and oxidant enzymes assay

Determination of superoxide dismutase activity

The superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972). The principle involves inhibition of epinephrine autoxidation in an alkaline medium at 480nm in a UV/VS via a spectrophotometer. The SOD activity was expressed in arbitrary units considering inhibition of autoxidation, as 1 unit of SOD specific activity.

Determination of catalase activity

The catalase activity was determined according to the method of Aebi (1984). The liver supernatant (0.5 mL) was mixed with 0.5 mL of 30 M of hydrogen peroxide, 1 mL of 6M H_2SO_4 and 7 mL of 2 mM of potassium permanganate. The absorbance of the mixture was read at 480 nm within 30 to 60 s against distilled water. The result was expressed in µmol/mg.

Determination of malondialdehyde

The method of Hunter *et al*., 1963, modified by Gutteridge and Wilkins, 1982 was adopted for the estimation of malondialdehyde (MDA) concentration in the liver. A 1 mL volume of TBA reagent and 0.5 mL of trichloroacetic acid (TCA) were mixed with 1 mL of liver supernatant. The mixture was heated at 60°C for 20 min in a water bath. The mixture was centrifuged at 400 rpm for 10 min. The absorbance of the supernatant was read at a wavelength of 540 nm. The result was expressed in nmol/mg protein.

Liver histology

Liver samples were fixed in 10% buffered neutral formalin and processed for embedding in paraffin. Sections of 5-6 µm thickness of the liver were placed on a microscope slide and stained

with hematoxylin and eosin. The stained slides were examined under a light microscope, and their micrographs were obtained.

Statistical analysis

Results are expressed as mean ± SEM for five animals per group. One-way analysis of variance (ANOVA) was used to assess the statistical significance of the data. Fisher's Least Significant Difference (LSD) test was used for post hoc analysis (Multiple comparisons). $P<0.05$ was considered significant.

RESULTS

***In-vitro* antioxidant activities of methanol extract of *C. lutea* root**

Table 1 showed the *in-vitro* antioxidant activities of methanol extract of *C. lutea* root. The phenol content in the extract was significantly lower ($P<0.05$) than the positive control (galic acid). The total antioxidant capacity of the methanol extract of *C. lutea* root was significantly higher ($P<0.05$) than the positive control (ascorbic acid). The IC_{50} of DPPH and metal chelating activities of the methanol extract of *C. lutea* root was significantly higher ($P<0.05$) than the positive control.

Table 1: *In-vitro* antioxidant activities of methanol extract of *C. lutea* root

Antioxidants	Standard (Control)	Methanol extract of *C. lutea root*
Phenol content (mg GAE/g)	99.7 ± 3.41	78.0 ± 3.10*
Total antioxidant capacity (mg AAE/g)	68.1 ± 2.31	81.0 ± 3.40*
DPPH IC_{50} (mg/mL)	1.4 ± 0.04	5.7 ± 0.19*
Metal chelating IC_{50} (mg/mL)	0.15 ± 0.004	1.40 ± 0.026*

*Showed significant difference at $P<0.05$ when compared with the control groups respectively.

Chemical compounds identified in methanol extract of *Carpolobia lutea* root

The GC–MS chromatogram of the methanol extract of *C. lutea* root showed twenty-one peaks indicating the presence of twenty-one chemical constituents. On comparison of the mass spectra of the constituents with the NIST library (Scarfone *et al*., 2008), the twenty-one constituents were characterised and identified. The compounds with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the methanol extract of *C. lutea* root are presented in Table 3. The results revealed that, nonanoic acid (12.6%) was found as the major component followed by naphthalene, 1,2,3,5,8,8a-hexahydro- (12.5%), octanoic acid (11.9%), n-hexadecanoic acid (7.8%), 9-octadecenoic acid methyl ester (7.0%) and hexadecanoic acid, methyl ester (7.0%).

Table 2: Chemical compounds identified in the methanol extract of *Carpolobia lutea* root

S/No	Retention time (min)	Name of compound	Molecular formula	Molecular weight	Peak area %
1.	6.83	Octanoic acid	$C_8H_{16}O_2$	144.2	11.9
2.	8.58	Nonanoic acid	$C_9H_{18}O_2$	158.2	12.6
3.	9.76	4-Ethyl-4methyl-1-hexene	C_9H_{18}	126.2	1.9
4.	11.29	2,5-cyclohexadiene-1,4-dione, 2,6-bis (1,1-dimethylethyl)-	$C_8H_8O_2$	136.1	0.7
5.	13.00	Hexadecane	$C_{16}H_{34}$	226.4	3.0
6.	14.28	Cyclohexene, 1-(1,1-dimethylethoxy)-3-methyl-	$C_{11}H_{20}$	168.3	3.0
7.	14.50	4-Bromo-2, 6-di-tert-butylphenol	$C_{14}H_{21}BrO$	285.2	2.4
8.	15.36	Octadecane	$C_{18}H_{38}$	254.5	1.6
9.	15.67	Propanal,dipropylhydrazone	$C_9H_{20}N_2$	156.3	1.8
10.	16.74	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	7.0
11.	17.22	n-Hexadecanoic	$C_{16}H_{32}O_2$	256.4	7.8
12.	17.57	Naphthalene, 1,2,3,5,8,8a-hexahydro-	$C_{26}H_{24}N_2O_2$	428.5	12.5
13.	18.35	Cycloeicosane	$C_{20}H_{40}$	280.5	4.0
14.	18.45	9,12-Octadecadienoic acid (Z,Z)-	$C_{19}H_{34}O_2$	294.5	2.4
15.	18.51	9-Octadecenoic acid, methyl ester,(E)-	$C_{19}H_{36}O_2$	296.5	7.0
16.	18.75	Methyl 16-methyl-heptadecanoate	$C_{19}H_{38}O_2$	298.5	3.2
17.	18.96	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.4	1.2
18.	20.19	Butylphosphonic acid, pentyl 4-(2-phenylprop-2-yl)phenyl ester	$C_{17}H_{14}O_2$	130.2	4.8
19.	20.43	Dimethyl 9-oxooctadecanedioate	$C_{20}H_{36}O_5$	356.5	4. 6
20.	20.95	Z,E-2-Methyl-3,13-octadecadie-1-o	$C_{19}H_{36}O$	280.5	3.6
21.	21.34	Octadecanamide	$C_{18}H_{37}NO$	283.5	3.3

Figure 1 showed that alkaline phosphatase activity was significantly increased (P<0.05) in cadmium-treated group when compared with the control group. There was a significant decrease (P<0.05) in 100 mg/kg and 200 mg/kg *C. lutea* treated groups when compared with control. It also showed that there was a significant decline P<0.05) in Cd+100 mg/kg and Cd+200 mg/kg *C. lutea* treated groups when compared with Cd-treated group.

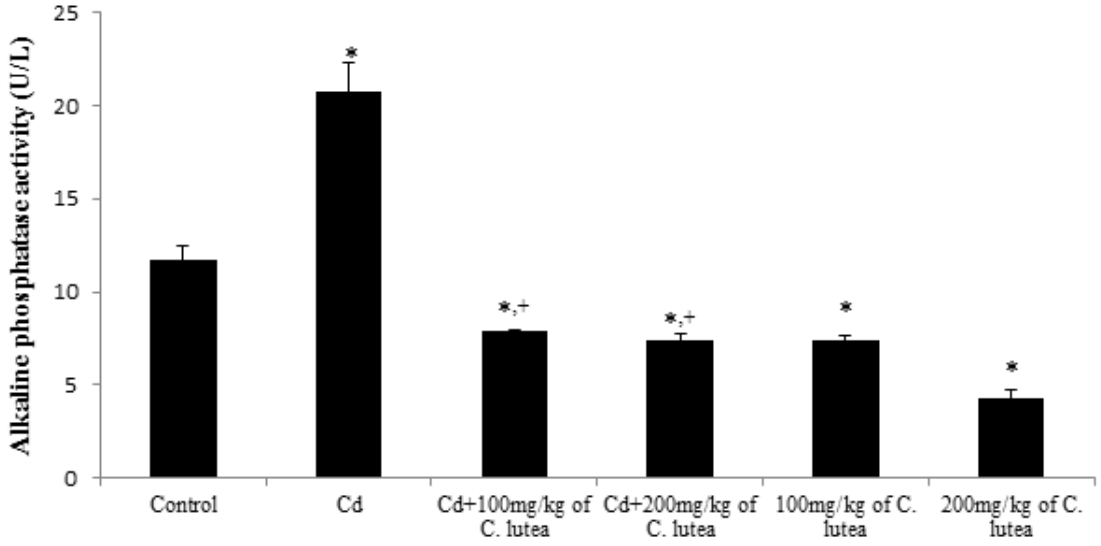

Figure 1: Effect of methanol extract of *Carpolobia lutea* root on liver alkaline phosphatase activity in male Wistar rats treated with cadmium

*,+ showed significant difference at p<0.05 when compared with the control and Cadmium groups, respectively

Figure 2 showed that alanine aminotransferase activity was significantly increased ($p<0.05$) in Cd-treated group when compared to control group, and a significant decrease ($p<0.05$) was observed in 100 mg/kg, and 200 mg/kg *C. lutea* treated groups when compared with the control.

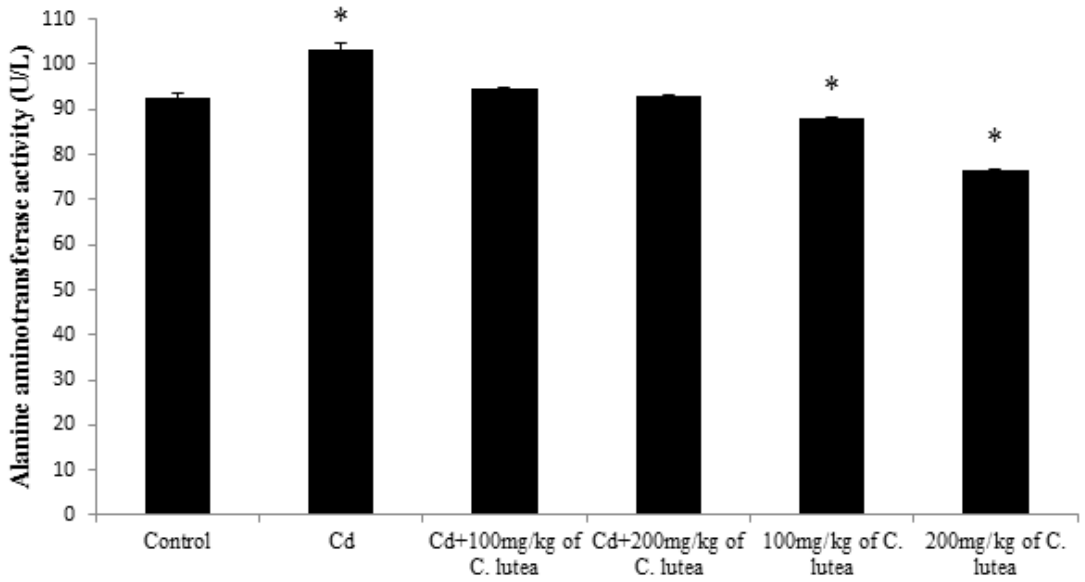

Figure 2: Effect of methanol extract of *Carpolobia lutea* root on liver alanine aminotransferase activity in male Wistar rats treated with cadmium

$*^{,+}$ showed significant difference at $P<0.05$ when compared with the control and Cadmium groups, respectively

The aspartate aminotransferase (AST) activity result in Figure 3 shows that there was significant increase ($P<0.05$) in Cd, Cd+100 mg/kg *C. lutea* and Cd+200 mg/kg *C. lutea* treated groups when compared with control, while a significant decrease ($P<0.05$) was observed in 100 mg/kg and 200 mg/kg *C. lutea* treated groups when compared with the control. Also, AST was significantly decreased ($P<0.05$) in Cd+100 mg/kg *C. lutea* and Cd+200 mg/kg *C. lutea* treated groups when compared with the Cd-treated group.

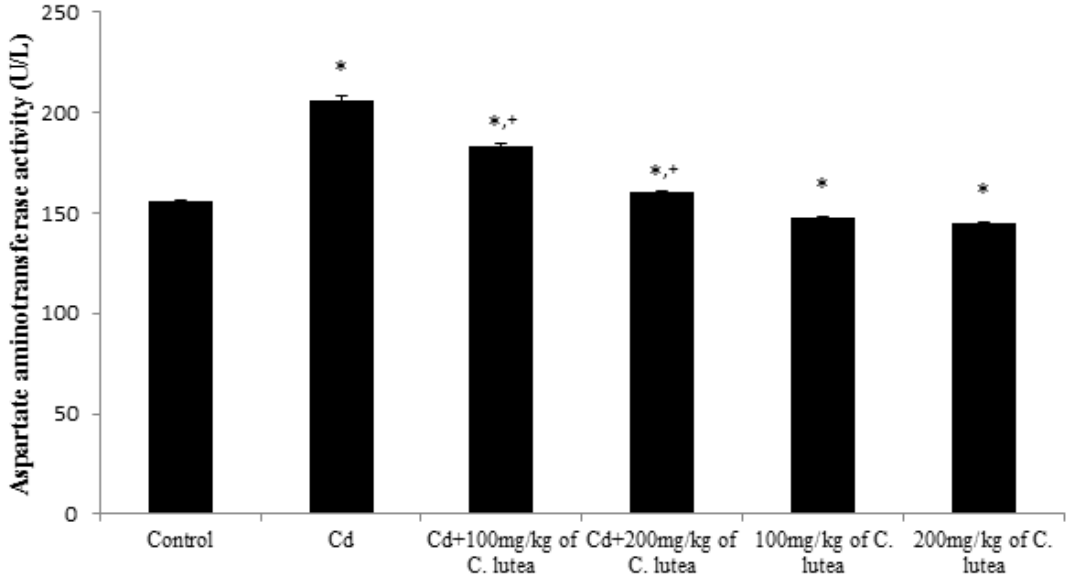

Figure 3: Effect of methanol extract of *Carpolobia lutea* root on liver aspartate aminotransferase activity in male Wistar rats treated with cadmium

*,+ showed significant difference at P<0.05 when compared with the control and Cadmium groups, respectively.

Figure 4 showed that superoxide dismutase activity was significantly reduced (P<0.05) in Cd and Cd+100 mg/kg *C. lutea* treated groups when compared with control. Also, there was significant increase (P<0.05) in Cd+200 mg/kg, 100 mg/kg *C. lutea* and 200 mg/kg *C. lutea* treated groups when compared with the control, while significant increase (P<0.05) was recorded in Cd+100 mg/kg *C. lutea* and Cd+200 mg/kg *C. lutea* treated groups when compared with the Cd group.

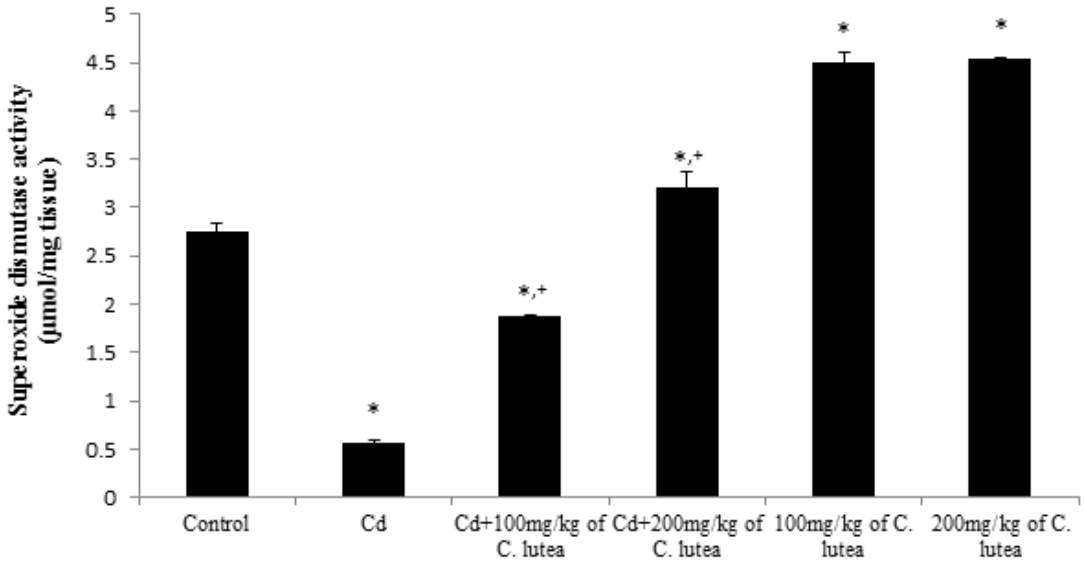

Figure 4: Effect of methanol root extract of *Carpolobia lutea* on liver superoxide dismutase activity in male Wistar rats treated with cadmium

*,+ showed significant difference at P<0.05 when compared with the control and Cadmium groups, respectively.

The result (Figure 5) showed that there was a significant reduction (P<0.05) in the catalase activity of cadmium-treated group when compared with the control. There was a significant increase (P<0.05) in 100 mg/kg *C. lutea* and 200 mg/kg *C. lutea* treated groups when compared with the control. Also, catalase activity was significantly increased (P<0.05) in Cd+100 mg/kg *C. lutea* and Cd+200 mg/kg *C. lutea* treated groups when compared with the cadmium group.

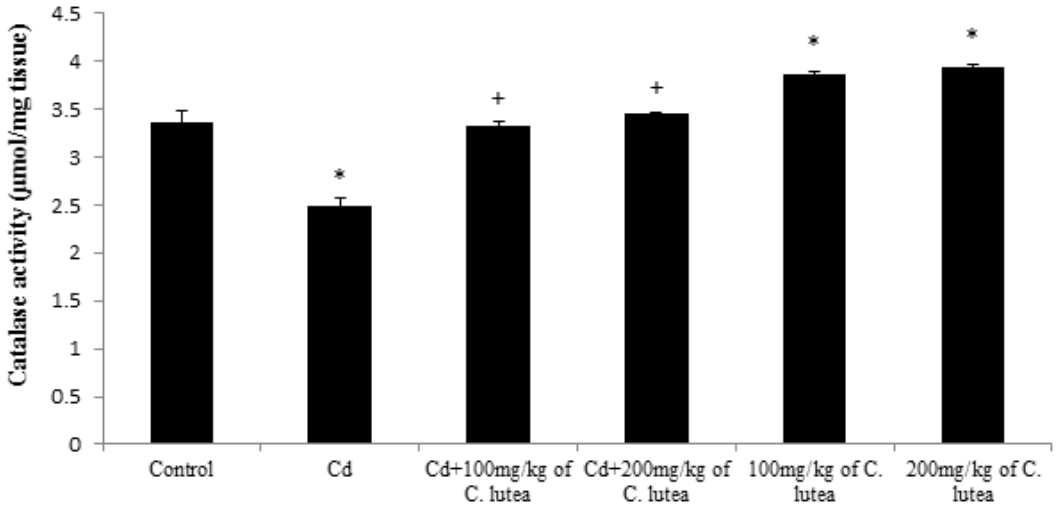

Figure 5: Effect of methanol extract of *Carpolobia lutea* root on liver catalase activity in male Wistar rats treated with cadmium

$*^{,+}$ showed significant difference at P<0.05 when compared with the control and Cadmium groups, respectively.

Figure 6 showed that malondialdehyde significantly increased (P<0.05) in Cd-treated group when compared with the control. Also, malondialdehyde significantly increased (P<0.05) in Cd+200 mg/kg *C. lutea*, 100 mg/kg *C. lutea* and 200 mg/kg *C. lutea* treated groups when compared with the control group. The malondialdehyde was significantly reduced (P<0.05) in Cd+100 mg/kg *C. lutea* and Cd+200 mg/kg *C. lutea* treated groups when compared with the Cd-treated group.

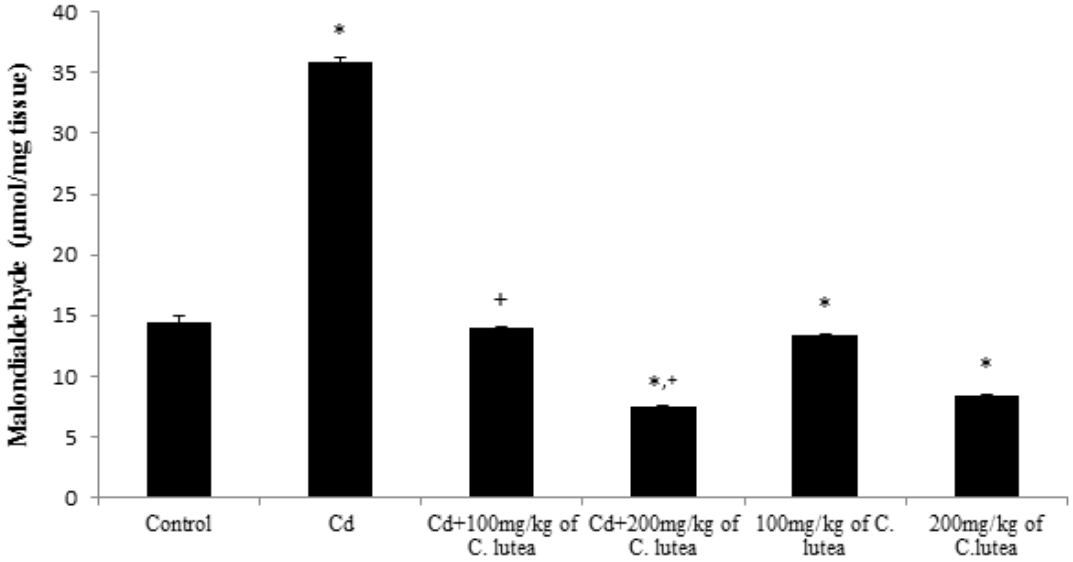

Figure 6: Effect of methanol extract of *Carpolobia lutea* root on liver malondialdehyde in male Wistar rats treated with cadmium

*,+ showed significant difference at P<0.05 when compared with the control and Cadmium groups, respectively.

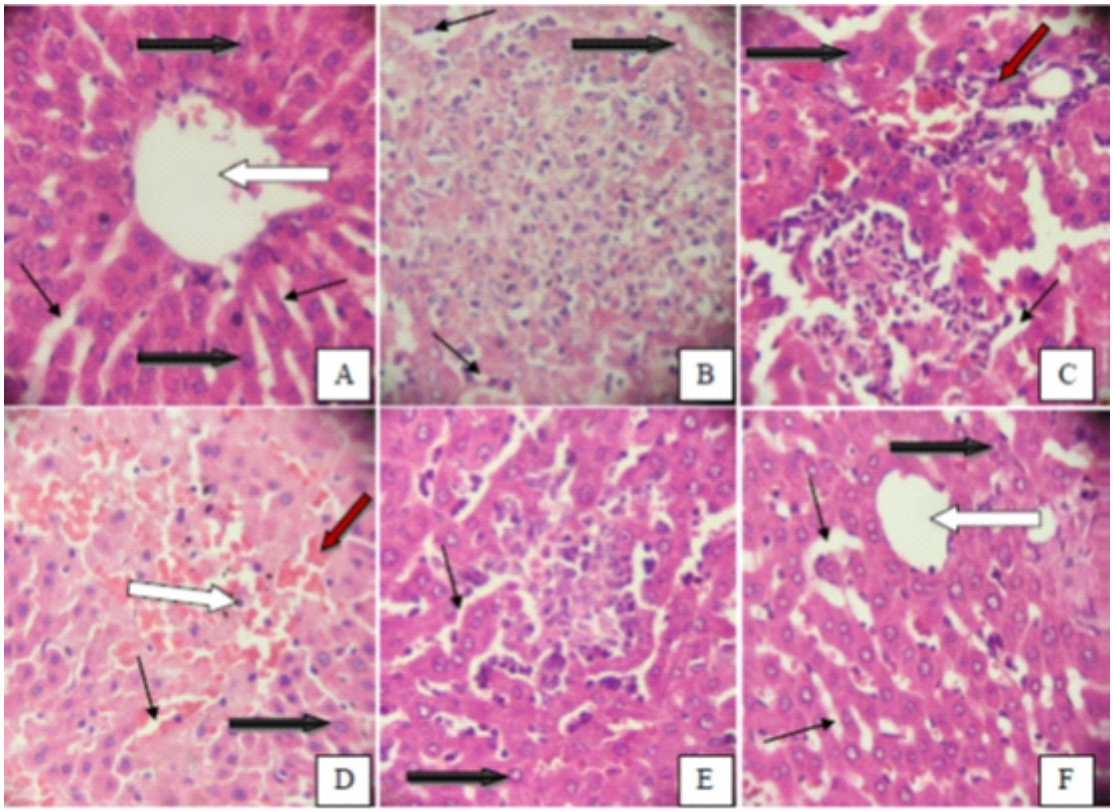

Plate 1: Effect of methanol extract of *C. lutea root* on liver histology in cadmium-treated male Wistar rats (×400)

(A): Control showed normal central venule (white arrow) and normal liver sinusoids (slender black arrow), hepatocytes generally appear normal (thick black arrow). (B): Cadmium group showed several inflammatory cells infiltrating the liver sinusoids (slender black arrow), the hepatocytes morphology appear normal generally (thick black arrow). (C): Cd+100mg/kg *C. lutea* group showed mild periportal infiltrating by inflammatory cells (thick red arrow), the sinusoids and hepatocytes morphology appears normal (slender black and thick black arrows respectively). (D): Cd+200mg/kg *C. lutea* group shows the moderate focal area of hemorrhage (thick white arrow), there is mild infiltration of the sinusoid and periportal by inflammatory cells (slender black arrow and thick red arrow respectively), the hepatocytes morphology appear normal (thick black arrow). (E) and (F): 100mg/kg *C. lutea* and 200mg/kg *C. lutea* show normal central venule (thick white arrow), the sinusoid and hepatocytes morphology (slender and thick black arrows respectively) appear normal.

DISCUSSION

The present study examined the possible protective role of methanol extract of *C. lutea* root against cadmium-induced changes in liver biochemical and antioxidant indices of male Wistar rats.

Previous studies by Schlesier *et al.* (2002) showed that when analyzing the antioxidant activity, it is preferable to use at least two parameters. In this study, four parameters were used to assess the antioxidant activity of methanol extract of *C. lutea*: DPPH radical scavenging activity, total antioxidant capacity (TAC), total phenolic content and metal chelating activities. The result of the study proved that the methanolic extract of *C. lutea* contains a considerably high total antioxidant capacity (TAC) and less phenolic content. The observed high total antioxidant capacity of *C. lutea* may be due to different antioxidant compounds detected in the extract. Phenolic compounds are ubiquitous secondary metabolites in plants. They are known to have antioxidant activity, and it is likely that the activity of these extracts is due to these compounds (Tepe *et al.,* 2006). It is well documented that the antioxidant activity of putative antioxidants has been attributed to various mechanisms. They include: prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging power (Gülçin *et al.,* 2003). Phenolic content from plant extracts have been found to correlate with radical scavenging activity (Sim *et al*., 2010). Polyphenolics have high redox potentials which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kahkonen *et al*., 1999). DPPH and metal chelating of the methanol extract of C. *lutea* root obtained in this study were considerably high compared to the standard. The DPPH assay has been used as a fast and useful marker to search for *in-vitro* antioxidant activity of pure compounds as well as extracts from plants (Ara and Nur, 2009). The DPPH antioxidant activity of the extract was estimated from the absorbance of the free radical. It was observed that antioxidant value of the extract is higher with significant difference to that of standard Gallic acid. This suggests the involvement of phenolic compounds in *C. lutea* extract with the reduction of DPPH (Aliyu *et al*., 2012). Metal chelating ability was significant as they caused reduction in concentration of catalyzing transition metal in lipid peroxidation (Duh *et al*., 1999).

In the present study, twenty-one compounds were identified from the methanol extract of the root of *C. lutea* by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The identified compounds found in *Carpolobia lutea* possess various therapeutic and medicinal values. Among the identified compounds are nonanoic acid, n-Hexadecanoic acid, Hexadecane, 4-Bromo-2,6-di-tert-butylphenol, Hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid (Z, Z)-, 9-Octadecenoic acid, methyl ester, 9,17-Octadecadienal, (Z), Butyl phosphonic acid and pentyl 4-(2-phenylprop-2-yl) phenyl ester. In previous studies, pentyl 4-(2-phenylprop-2-yl) phenyl ester was reported to have antioxidant, anti-inflammatory, antitumor and anti-carcinogenic activities respectively (Zhiwei *et al*., 2013; Arockia and Veerabahu, 2014).

The activities of ALP, ALT and AST were evaluated to examine the toxic effect of the cadmium on rat liver. Gaskill *et al.* (2005) reported

severe hepatic toxicity as shown by significant increases in liver enzymes (ALT, AST, ALP) in Cd-treated group as compared with the control group which is similar to our observation in this study. Lakshmi *et al.* (2012) also affirmed the results obtained by revealing that Cd exposure elevated the levels of hepatic biomarkers. The administration of *C. lutea* in Cd-treated rats significantly reduced the activities of ALP and AST, possibly due to the antioxidant activities of methanol extract of *C. lutea* as reported by Akinola *et al.* (2020). This study also revealed that *C. lutea* root extract contain phytochemicals like alkaloids, tannins, saponins, anthraquinones, flavonoids, cardiac glycosides, terpenes and simple sugar, which are known to have antioxidant properties.

In a healthy body, reactive oxygen species (ROS) and antioxidants remain in balance. When the balance is disrupted toward an excess of ROS, oxidative stress occurs. This phenomenon results from an imbalance between pro-oxidants (free radical species) and the body's scavenging ability (antioxidants) (Agarwal *et al.*, 2005). Reactive oxygen species not only serve as key signal molecules in physiological processes but also have a role in pathological processes. Results from this study show that cadmium increased hepatic malondialdehyde which is a marker of lipid peroxidation, reduced hepatic superoxide dismutase and catalase activities. The results are in accordance with the findings of Eybl *et al.* (2004) suggesting that cadmium-induced liver toxicity can cause oxidative stress and also lipid peroxidation. This experimental evidence indicates the involvement of oxidative stress in cadmium-mediated liver damage. As shown previously, oxidative stress (enhanced ROS and LPO as well as altered anti-oxidant enzymes activities) is considered as one of the underlying mechanisms of cadmium-induced toxicity (Ikediobi *et al.*, 2004) and antioxidants have been successful in abating some of these deleterious effects (Yadav and Khandelwal, 2008). Several studies have suggested that cadmium causes oxidative stress and induces oxidative damage by disturbing the antioxidant defence systems (Waisberg *et al.*, 2003).

The methanol extract of *C. lutea* root improved the observed effects of cadmium-induced elevation of MDA and reduction of SOD and catalase activities in the liver. Under normal situations, the natural defence system including the endogenous antioxidant enzymes like catalase, glutathione peroxidase and superoxide dismutase, are among the primary antioxidant system in cells responsible for the deactivation of ROS and protecting against oxidative damage. Superoxide dismutase is a ubiquitous cellular enzyme that dismutates superoxide radical to H_2O_2 and oxygen and one of the chief cellular defense mechanisms (Irmak *et al.*, 2002). The toxic effect of H_2O_2 formed by SOD and other processes is prevented by catalase present in the peroxisomes of nearly all aerobic cells, which scavenged the hydrogen peroxide by catalyzing its decomposition into water and molecular oxygen without the production of free radicals. Therefore, the statistically significant dose-dependent increase in the activity of hepatic SOD and catalase activities in 100 mg/kg and 200 mg/kg *C. lutea* treated rats may be suggestive of the inherent antioxidant activities of the secondary metabolites of the extract and their ability to improve the antioxidant status in animals. *Carpolobia lutea* extract ameliorated cadmium-induced oxidative damage by improving antioxidant defense systems through increases in SOD, CAT and reduction of MDA. This effect of MCL is due to its antioxidant potentials as we earlier explained (Akinola *et al.*, 2020). Accordingly, we showed that *C. lutea* root extract contain phytochemicals like alkaloids, tannins, saponins, anthraquinones, flavonoids, cardiac glycosides, terpenes and simple sugar all of which are known to have antioxidant properties.

Cadmium was shown to provoke some histopathological changes in the liver of cadmium-treated rats. The changes observed in the liver include infiltration of cell aggregate and infiltration of liver sinusoids by the inflammatory cell. These destructions are also in agreement with the earlier works of Habeebu *et al.* (2000) in which they showed that cadmium caused liver damage. The observed histopathological liver damage in cadmium-treated rats may be supported by the elevated of liver damage biomarkers (ALP, ALT and AST activities) in this study. Co-administration of low and high doses of methanol extract of *C. lutea* significantly attenuated the histopathological alterations of the liver rats in a

dose-dependent fashion. The beneficial effect of the methanol extract of *Carpolobia lutea* root may be due to its antioxidant properties which may be attributed to the presence of essential phytochemicals like alkaloids, tannins, saponins, anthraquinones, flavonoids, cardiac glycosides, terpenes and simple sugar, which are known to have antioxidant properties (Yakubu and Jimoh, 2015; Akinola *et al*., 2020).

In conclusion, the results obtained in the present study indicate that this plant has potential as a natural source of antioxidants, capable of protecting against cadmium-induced liver damage and other free radical-mediated diseases.

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