integrity Research Journals

Journal of Bioscience and Biotechnology Discovery
Volume 8(5), pages 122-135, October 2023
Article Number: 3A4E66141
ISSN: 2536-7064
https://doi.org/10.31248/JBBD2023.192
https://integrityresjournals.org/journal/JBBD

Full Length Research

Ameliorating effect of *Calotropis procera* methanol leaf extract against oxidative stress and lipid peroxidation induced by fipronil in Wister albino rats

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Received 14th August 2023; Accepted 20th September 2023

ABSTRACT: The exposure of humans to oxidative stress inducing chemicals is on the increase due to global industrialization. It has become vital to source for solution by searching for medicinal plants with antioxidant potential. This study was carried out to evaluate the toxicological effect and the antioxidant potential of *Calotropis procera* in fipronil induced Wister Albino rats. Qualitative and quantitative phytochemical and antioxidant screenings of the leaf extract were conducted using standard laboratory protocols. Acute oral toxicity was conducted using Lorke's method to determine the LD_{50} of the plant extract. The sub-chronic toxicity was evaluated using the Organisation for Economic and Cultural Development method. The *in vivo* antioxidant potential of the plant extracts was determined using standard methods. Qualitative phytochemical analysis revealed the presence of saponins, flavonoids, phenols, cardiac glycosides, steroids, terpenoids and coumarines. Quantitative analysis revealed the amounts of saponins (32.67%), cardiac glycosides (7.6 mg/g), phenols (28 mg GAE/g), flavonoids (7.37 mg/g) and steroids (21.53 µg/mg) that were found. The LD_{50} was calculated to be 2154 mg/kg. Sub-chronic toxicity studies revealed insignificant ($p>0.05$) increases in body weight. Insignificant ($p>0.05$) changes in haematological parameters were also observed across treated groups compared to normal control. Liver and kidney function parameters were significantly ($p<0.05$) altered at higher (dose) of extract compared to normal control. *In vivo* antioxidant activity of the extract revealed strong antioxidant potentials due to significant ($p<0.05$) alteration of enzymatic (SOD, CAT, GSH and MDA) and non-enzymatic (vit C and E) antioxidants in the extract treated groups when compared to fipronil treated and normal control groups. Histological screening revealed that the liver and kidney tissues were significantly ($p<0.05$) altered at higher (dose) of extract administration. In conclusion, the present study validates the antioxidant potential and hence ameliorating effect of *C. procera* methanol leaf extract from toxic effects of phenyl pyrazole insecticides.

Keywords: Antioxidant, *Calotropis procera,* fipronil, toxicity.

INTRODUCTION

Plants have been identified as one of the most essential sources of medicinal agents for centuries and quite a large number of ground breaking drug components which have been isolated from natural plant sources, have made large contributions to human health and well-being. They have been used since medieval times to alleviate ailments (Gallo *et al.,* 2020).

Calotropis procera, also known as the apple of Sodom, is a species of flowering plant in the family Asclepiadaecae that is native to North Africa, Tropical Africa, and Western and South Asia (Merzaia *et al.,* 2017). It is a shrub with having simple stem with mainly huge and a corky back. It oozes a sticky white latex when its leaves or stem is cut or broken. It grows in tropical and arid regions as it is resistant to drought. It could grow up to 5.5 m in height. It has a variety of biological benefits which include anti-proliferative, anti-inflammatory as well as wound healing activity (Tsala *et al*., 2015).

The imbalance between the number of Reactive Oxygen Species (ROS) produced and the antioxidants acting as

defence in the body creates a condition which has far reaching health implications. If there is too much ROS produced and too few antioxidants in the body for protection, a condition called oxidative stress develops (Gwozdzinski *et al.,* 2021). Oxidative stress is hence the cytotoxic consequence of oxyradial and oxidant formation and the reaction of such formation with constituents of the cell (Tada and Suzuki, 2016). Previous studies have reported that pesticides modify antioxidants and elicit oxidative stress in animals which were investigated as a potential mechanism of pesticide toxicity (Alipanah *et al.,* 2022).

Fipronil is a pesticide belonging to the phenylpyrazole class of pesticides, widely used in veterinary medicine and agriculture to control bugs, ticks, fleas, locusts and ants found on pets and livestock. Due to exposure of humans, pets and livestock to these insecticides, contamination of food, water and air is currently a major public health concern as It has been reported to elicit oxidative stress in animals (De Barros *et al.,* 2017).

Exposure to phenylpyrazole is a universal public health concern, and this concern has increased due to increased exposure to these pesticides. The increase in exposure is in turn, due to their broad use and toxicity which is released into the environment (Gatto *et al.,* 2016). The use of plants to treat illnesses has always been the cheaper and more available alternative.

Thus, this work was carried out to evaluate the toxicity of *Calotropis procera* extract and its antioxidant potential against fipronil induced oxidative stress and lipid peroxidation in Wister Albino rats.

MATERIALS AND METHODS

Chemicals

Fipronil (Insecto SC 5%), Methanol, Chloroform, Ferric chloride (BDH chem. Company poled), Hydrochoric acid, Ammonia solution and Folin-Dennis reagent were manufactured by Qualikems in India, Sodium chloride used was manufactured by Avis chem, India. Ethanol, Nitric acid, Wagner's reagent, Fehling solution and Sodium hydrogen phosphate were manufactured by BDH chem. Company poled, England. All chemicals and reagents used in the study were of analytical grade.

Plant collection

Calotropis procera leaves were collected from Badariya in Birnin Kebbi Local Government Area of Kebbi State. The plant sample was authenticated by a Taxonomist from the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aleiro, Kebbi State, Nigeria. A voucher specimen numbered (KSUSTA/PSB/H/VOUCHER NO: 03) was given to the plant and deposited in the herbarium of the same department for referencing.

Preparation of extract

The leaves of the *Calotropis procera* plant were washed and allowed to dry at room temperature for 2 weeks. They were then macerated to a coarse powder using laboratory mortar and pestle. Five hundred grams (500 g) of the powdered sample was soaked in 2500 ml of methanol for 48 hours and the mixture was filtered using muslin cloth and filter paper (Dupont *et al.,* 2002). The filtrate was then evaporated using an oven set at 45^{o}C. The *Calotropis procera* leaves sample yielded 59.76 g dry extract. The percentage yield of the extract was determined using the expression:

$$\% \text{ yield} = \frac{\text{Weight of dried concentrated extract}}{\text{Weight of ground plant material}} x\ 100$$

Experimental animals and design

A total of 57 Wistar Albino rats weighing 100 to 140 g were obtained from the animal house of Zubai Animal Research Centre, Aliero, Kebbi State, Nigeria. They were housed in plastic cages and allowed to acclimatise for seven (7) days with free access to standard animal pellet diet and water *ad libitum*. Ethical approval was given by the Ethical Committee of the Kebbi State University of Science and Technology, Aliero. Animal handling was done in compliance with standard protocols.

Phytochemical analysis

Qualitative determination of phytochemicals was carried out in accordance with the methods of Soforowa (1983), Gawron-Gzella (2012), Trease and Evans (2002), Ekwueme *et al.* (2015), Madhu *et al.* (2016) and Sisodiya and Shrivastava (2018).

Qualitative phytochemical analysis

Test for alkaloids

One (1) ml of *C. procera* plant extract was added to 1 ml of potassium iodide (Wagner's reagent) and the resulting solution was shaken. The appearance of a reddish-brown precipitate signified the presence of alkaloids (Soforowa, 1983).

Test for saponins

To 0.5 g of the extract, 2.5 ml of distilled water was added and shaken vigorously. The appearance of froth that lasts for several minutes indicates the presence of saponins (Ekwueme *et al.,* 2015).

Test for flavonoids

To 2 ml of the extract, a few drops of concentrated sodium hydroxide was added. An intense yellow colour was observed. Then few drops of hydrochloric acid were added and the yellow colouration disappeared. The emergence of pink colouration indicates the presence of flavonoids (Ekwueme *et al.,* 2015).

Test for phenols

Determination of phenols was conducted according to the method described by Gawron-Gzella (2012). Two millilitres (2 ml) of extract was added to 2 ml of ferric chloride solution. A deep bluish-green solution indicates the presence of phenols.

Test for cardiac-glycosides

To 0.5 g of the extract, 2 ml of 3.5% ferric chloride solution was added and allowed to stand for a minute. And then 2 ml of conc. H_2SO_4 was carefully poured down the wall of the tube so as to form a lower layer. The presence of a reddish-brown ring at the lower chloroform level indicates the presence of cardiac glycoside (Trease and Evans, 2002).

Test for steroids

One (1) ml aqueous *C. procera* crude extract was put in a test tube. Then 2 ml of chloroform and concentrated H_2SO_4 were added. The appearance of red colour at the lower chloroform layer signified the presence of steroids (Trease and Evans, 2002).

Test for terpenoids

A mass of extract (0.5 g) was mixed with chloroform (2 mL) and filtered. Three drops of concentrated H_2SO_4 were then carefully added to the filtrate, and a thin reddish-brown layer indicated the presence of terpenoids (Soforowa, 1983).

Test for anthraquinones

Exactly 2 ml of the dilute extract was shaken with 10 ml of benzene, and 5 ml of 10% ammonia solution was added. The mixture was stirred and the presence of pink, red or violet colour in the ammoniacal (lower) phases indicates the presence of anthraquinones (Soforowa, 1983).

Test for coumarines

To 2 ml of extract-solution, a few drops of alcoholic NaOH were added. Yellow colouration indicates the presence of coumarin (Soforowa, 1983).

Test for xanthoproteins

A few drops of concentrated HNO_3 were added to 1 ml of the test sample and mixed properly. Thereafter, 2 ml of ammonia solution was added. The formation of a red precipitate indicates the presence of xanthoprotein (Trease and Evan, 2002).

Quantitative phytochemical analysis

Analysis to estimate the contents of some phytochemicals found was carried out. Saponins, flavonoids and steroids were done according to methods described by Madhu *et al.* (2016). Phenolic contents were determined in accordance with the methods by Gawron-Gzella (2012) and Cardiac-glycosides contents were estimated using the methods by Sisodiya and Shrivastava (2018).

Acute toxicity

The acute toxicity (LD_{50}) value of the *Calotropis procera* methanol leaf extract was carried out using standard procedures described by Lorke (1983).

Sub-chronic toxicity

Sub-chronic toxicity was carried out according to the OECD (2008) method. Fifteen (15) rats were distributed into five groups of three rats each and were treated as follows: The first group of rats served as the normal control group and received 5 ml/kg of distilled water only. The second group received 108 mg/kg body weight of *Calotropis procera* leaf methanol extract. Rats in the third, fourth and fifth groups were administered 215 mg/kg, 323 mg/kg and 431 mg/kg b.wt of the plant extract respectively for 28 days. Change in body weight was monitored and recorded weekly. The rats were equally closely monitored for changes in behaviour such as restlessness, salivation, loss of fur, changes in sleep pattern and mortality throughout the experimental period. The rats were fasted overnight at the end of the experimental period, anaesthetized using chloroform and then sacrificed. Blood samples were collected in non-heparinised bottles for biochemical screening and heparinized bottles for haematological analyses, while organs such as the liver and kidney were used for histopathological evaluation.

Administration of fipronil

Twenty (20) rats were divided into four groups of five rats each and administered fipronil (10 mg/l) in water to induce oxidative stress and lipid peroxidation. The concentration of fipronil that was used in this study represents 2.0 mg/kg b.wt of fipronil based on the average consumption of water

and body weight of treated rats. Signs of oxidative stress induction such as weakness, salivation and loss of fur were exhibited by rats in the treated groups (Al-Badran, 2019).

In vivo antioxidant assay

The antioxidant assay was carried out following the repeated dose method by OECD (2008). Experimental thirty rats were divided into six groups of five rats each. The first group received only water and served as the normal control. The second group received 0.2 mg/kg body weight of the standard drug, vitamin C and served as the positive control group. The third group received 10 ml/kg of fipronil and served as the negative control group. The fourth, fifth and sixth groups were administered 100 mg/kg, 200 mg/kg and 400 mg/kg of *Calotropis procera* leaf extract respectively and 10 ml/kg of fipronil for 45 days. The rats were sacrificed at the end of the experimental period. Blood samples were then collected for analysis of biochemical parameters such as AST and ALT. The liver tissue was cropped immediately after sacrifice and cleaned in saline solution. It was then homogenised in 10% (w/v) 100 mM phosphate buffer with a pH of 7.4. The liver homogenate was then centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was then obtained for analysis of oxidative stress parameters such as CAT, SOD, GSH and MDA.

Collection of blood

This was carried out in accordance with Abidemi *et al.* (2017). At the end of the experimental period, the rats were fasted overnight. They were then anaesthetized with chloroform in accordance with animal ethics and sacrificed by cervical dislocation. The blood samples were collected using coagulant and anticoagulant sample bottles. The samples were then centrifuged at 4000 rpm and the serum was obtained. Blood samples were then collected for analysis of biochemical parameters such as ALP, AST and ALT.

Determination of haematological parameters

Automated haematological analyzer Sysmex XS800i (Sysmex corporation, USA) was used to analyze all the haematological parameters using the reagents ALFA-Lyse and diluents according to Theml *et al*. (2004). A full blood count (FBC) was carried out.

Determination of serum biochemical parameters

The biochemical analysis of serum samples was performed using reagent kits (Randox commercial Kits). Biochemical parameters measured were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, direct and total bilirubin and total protein (TP). They were determined according to methods described by Eseyin *et al.* (2006).

Oxidative stress biomarkers

Superoxide Dismutase (SOD)

The SOD was determined according to the method of Xin *et al.* (1991). The method is based on the SOD enzyme's ability to inhibit phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye and the absorbance was read at 560 nm for 5 minutes and expressed as umol/g protein.

Catalase (CAT)

Catalase activity was determined using the method of Aebi (1984). This method is based on the catalase decomposition of H_2O_2. The sample with catalase is incubated in the presence of a known concentration of H_2O_2. The absorbance is measured at 570 nm. CAT activity was expressed as µmol/g protein.

Lipid Peroxidation (LPO)

The measure of lipid peroxidation was carried out according to the method of Wallin *et al.* (1993). The MDA values were expressed as µmol/g of protein.

Histological analysis

Immediately after sacrifice, at the end of the subchronic toxicity test period, the liver tissues of the animals were removed and fixed in 10% formalin as described by Kiernan (2000).

Statistical analysis

The data generated from the study were presented as Mean ± Standard error of mean (SEM) and subjected to one-way analysis of variance (ANOVA). The statistical difference between means was determined using Duncan multiple comparison test using statistical package for social science (SPSS) version 20. Values are considered statistically significant at $p<0.05$.

RESULTS

Percentage yield

The percentage extract of *Calotropis procera* methanol leaf sample was 11.95%. The extract easily dissolves in water and is dark green in colour; with a gummy texture.

Table 1. Qualitative phytochemical constituents of *Calotropis procera* leaf methanol extract.

Phytochemical	Result
Saponins	+
Flavonoids	+
Alkaloids	ND
Tannins	ND
Phenols	+
Cardiac Glycosides	+
Steroids	+
Terpenoids	+
Quinones	ND
Coumarins	+
Xanthoproteins	ND

Key: + = Present, ND = Not detected.

Table 2. Quantitative phytochemical composition of the plant extracts.

Phytochemicals	*C. procera* methanol leaves extract
Saponins (%)	32.67±0.49
Flavonoids (mg QE/g)	7.37±0.06
Phenols (mg GAE/g)	28.00 ±0.20
Steroids (µg/mg)	21.53 + 0.31
C. glycosides (mg/g)	7.60 + 0.25

Values are presented as mean± SEM of triplicate value.Mg/g = milligram/gram, mgQE/mg = Quercetin Equivalent/gram and GAE/g = Garlic acid equivalent/gram.

Phytochemical screening

Qualitative phytochemical composition

The qualitative phytochemical screening of *Calotropis procera* leaf methanol extract is presented in Table 1. It revealed the presence of saponins, flavonoids, phenols, cardiac glycosides, steroids, terpenoids and coumarines while alkaloids, tannins, quinones and xanthoproteins were not detected.

Quantitative phytochemical composition

The quantitative phytochemical screening of *Calotropis procera* methanol leaves extract revealed the amounts of saponins (32.67%), phenols (28 GAE/g), steroids (21.53 µg/mg), cardiac glycosides (7.60 mg/g) and flavonoids (7.37 mg/QE) that were found (Table 2).

Acute toxicity (LD_{50}) profile of *C. procera* methanol leaf extract

The LD_{50} of the extract was calculated to be 2154 mg/kg. There were signs of toxicity and mortality recorded during the fourteen days of observation after treatment.

Effect of *Calotropis procera* extract on body weight

The effect of *C. procera* methanol leaf extract on the body weight of animals is shown in Figure 1. A progressive increase in body weight was observed across all groups throughout the experimental period. This increase was steeper in rats given the highest dosage.

Effect of *Calotropis procera* extract on liver biomarkers

The effect of *C. procera* methanol leaf extract on liver function revealed a significant ($p<0.05$) increase in AST, ALB and DB only in the 431 mg/kg treated group compared with normal control. While ALT and ALP revealed significant ($p<0.05$) increases in all extract treated groups compared to normal control. TP revealed significant ($p<0.05$) increases only in groups treated with 323 mg/kg and 431 mg/kg compared to normal control. However, TP revealed significant increases in groups treated with extract 215 mg/kg and 431 mg/kg compared with normal control (Table 3).

Effect of *Calotropis procera* methanol extract on biomarkers of kidney function in albino rats

The results for the effect of *C. procera* methanol leaves extract revealed a significant ($p<0.05$) increase in Na^+ in all extract treated groups compared to normal control. However, there was no significant ($p>0.05$) difference in K^+ in normal control, and extract treatment 108 mg/kg while a significant ($p<0.05$) increase in K^+ was observed in extract treatment 215 mg/kg, 323 mg/kg and 431 mg/kg compared to normal control. There was significant ($p<0.05$) decreases in Cl^- in all extract treatment compared to normal control. HCO_3 revealed non-significant ($p<0.05$) differences in all extract treated groups compared to normal control. Urea and creatinine biomarkers revealed significant increases ($p<0.05$) in all extract treated groups compared to normal control (Table 4).

Effect of *Calotropis procera* methanol extract on haematological indices in Albino rats

The results for the effect of *C. procera* methanol leaves extract on haematological indices revealed a significant ($p<0.05$) increase in white blood count (WBC), lymphocytes (LYM) and platelets (PLT) only in groups treated with extract 108 mg/kg compared to normal control respectively. While granulocytes revealed significant ($p<0.05$) increases only in groups treated with the highest

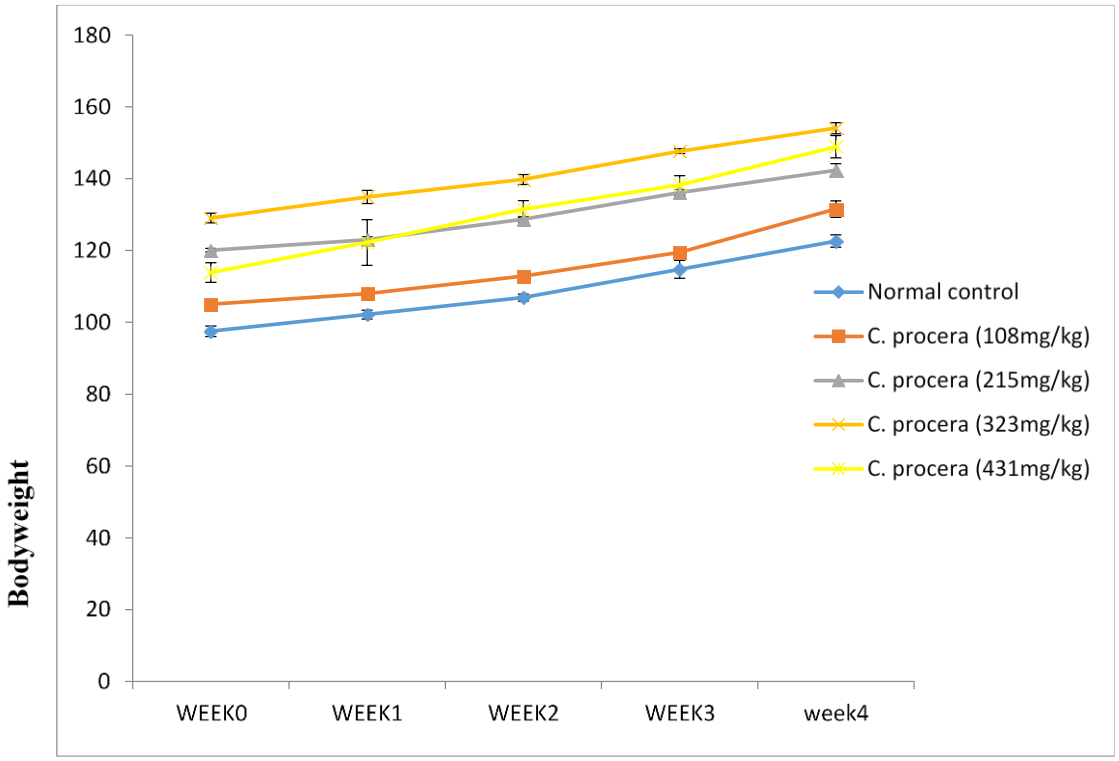

Figure 1. Bodyweight of animals administered with methanol leaf extract of *Calotropis procera* for four weeks {Group 1(normal control), group 2 (108mg/kg b.wt), group 3 (215mg/kg b.wt), group 4 (323mg/kg b.wt) and group 5 (431mg/kg b.wt)}.

Table 3. Effect of *Calotropis procera* methanol leaves extract on biomarkers of liver function in albino rats.

Parameter	Normal control (5 ml/kg)	*C. procera* extract (108 mg/kg)	*C. procera* extract (215 mg/kg)	*C. procera* Extract (323 mg/kg)	*C. procera* extract (431 mg/kg)
AST (U/L)	80.00±10.00^{a}	75.67±0.33^{a}	120.00±0.36^{ab}	111.67±0.88^{ab}	150.00±20.00^{b}
ALT (U/L)	16.67±0.33^{a}	91.67±0.33^{c}	88.33±5.67^{c}	61.67±0.33^{b}	76.00±9.45^{bc}
ALP (U/L)	40.70±0.09^{a}	41.83±2.27^{b}	42.29±0.07^{b}	47.10±0.21^{c}	49.17±0.19^{d}
ALB (G/l)	37.48±0.09^{a}	37.53±0.19^{a}	37.70±0.10^{a}	37.66±0.23^{a}	38.36±0.30^{b}
TP (G/l)	47.51±0.67^{a}	48.59±0.61^{ab}	48.74±0.47^{ab}	49.41±0.16^{b}	49.66±0.33^{b}
TB (mg/dL)	0.94±0.01^{a}	1.00±0.06^{a}	1.31±0.01^{b}	1.87±0.01^{c}	2.25±0.04^{d}
DB (mg/dL)	0.43±0.00^{a}	0.67±0.01^{a}	0.72±0.01^{a}	0.69±0.29^{a}	1.15±0.03^{b}

Values are presented as mean ± SEM (n = 3). Values with different superscripts along the rows were statistically significantly different at p<0.05AST- Aspartate Amino Transferase, ALT- Alanine Amino Transferase, ALP- Alkaline Phosphatase, ALB- Albumin, TP- Total Protein, TB- Total Bilirubin and DB- Direct Bilirubin.

Table 4. Effect of *Calotropis procera* methanol extract on biomarkers of kidney function in albino rats.

Parameter	Control D.H_2O (5 ml/kg)	*C. procera* extract (108 mg/kg)	*C. procera* extract (215 mg/kg)	*C. procera* extract (323 mg/kg)	*C. procera* extract (431 mg/kg)
Na^+ (mmol/l)	139.67±0.88^{a}	145.00±2.52^{b}	165.03 ±0.58^{c}	188.33±0.33^{d}	234.00±0.58^{e}
K^+ (mmol/l)	0.53±0.01^{a}	0.53±0.03^{a}	0.71±0.02^{b}	0.74±0.01^{bc}	0.77±0.03^{c}
Cl^- (mmol/l)	62.00±0.58^{e}	55.00±1.14^{c}	48.03±1.42^{b}	45.67±0.33^{b}	42.00±0.58^{a}
HCO_3^-(mmol/l)	89.63±67.18^{d}	24.20±0.35^{a}	27.17±0.20^{b}	27.20±0.06^{b}	28.90±0.25^{c}
Urea (mmol/l)	2.10±0.06^{a}	2.70±0.06^{b}	3.17±0.15^{c}	3.57±0.12^{d}	4.07±0.09^{e}
Creatinine (mg/dl)	0.86±0.03^{a}	1.23±0.07^{b}	1.53±0.03^{c}	1.57±0.03^{c}	1.63±0.03^{c}

Values are presented as mean ± SEM (n = 3). Values having the same superscript are not significantly different at (P>0.05) using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. Potassium (K^+), Sodium (Na^+), Bicarbonate (HCO^-_3).

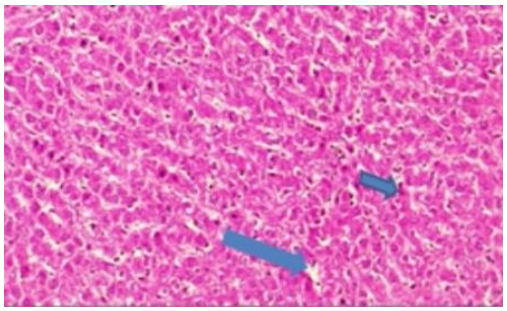

Plate 1. Photomicrograph of rat's liver obtained from control.

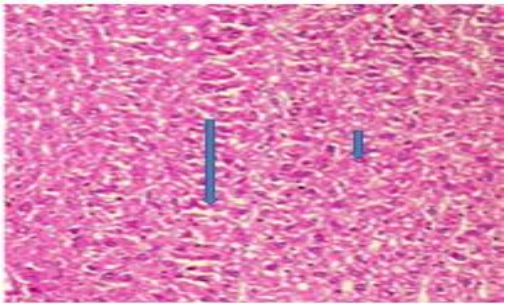

Plate 2 Photomicrograph of rat's liver obtained from group administered with 108mg/kg of *C. Procera* methanol leaf extract.

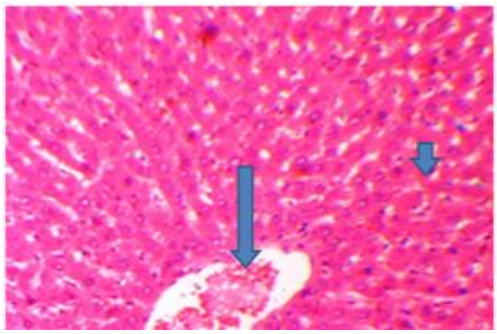

Plate 3 Photomicrograph of rat's liver obtained from group administered with 215mg/kg of *C. Procera* methanol leaf extract.

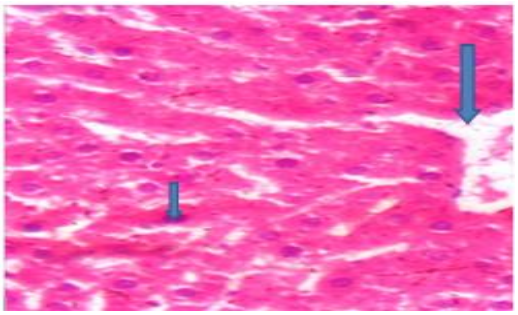

Plate 4 Photomicrograph of rat's liver obtained from group administered with 323mg/kg of *C. Procera* methanol leaf extract.

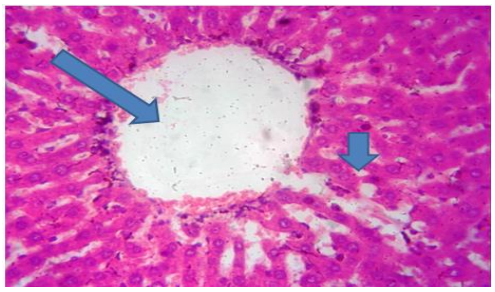

Plate 5. Photomicrograph of rat's liver obtained from group administered with 431mg/kg of *C. Procera* methanol leaf extract.

NOTE: Plate 1: showed normal portal triad (long arrow) and normal hepatocytes arrange in codes (short arrow). Plate 2: showed normal portal triad (long arrow) and normal hepatocytes arrange in codes (short arrow). Plate 3: showed congested central vein (long arrow), and normal hepatocytes (short arrow). Plate 4: Section showed hypertrophied central vein, (long arrow), and mild inflammation of hepatocytes (short arrow). Plate 5: showed inflamed hepatocytes (short arrow) and hypertrophied central vein (long arrow). Plates 1 and 2 are stained with H and E X 100 magnification. Plates 3, 4 and 5 are stained with H and E X 400 magnification.

dose of extract (431 mg/kg) compared to normal control. While red blood count (RBC) significantly ($p<0.05$) increases at groups treated with 215 mg/kg, 323 mg/kg and 431 mg/kg extract concentrations compared to the control respectively. However, there was no significant ($P>0.05$) difference in hemoglobin (HGB), HCT, MCH, MCHC and PCT in all extract treated groups compared to normal control (Table 5).

Effect of *Calotropis procera* methanol leaf extract on liver and kidney tissues

The subchronic effect of *C. procera* methanol leaf extract on liver histology showed a normal portal triad (long arrow) and normal hepatocytes arranged in codes in normal control (plate 1). Also, the group administered extract 108 mg/kg (plate 2) showed normal portal triad (long arrow)

Table 5. Effect of *Calotropis procera* methanol extract on hematological indices in albino rats.

Parameter	Control D.H_2O 5 ml/kg	*C. procera* extract (108 mg/kg)	*C. procera* extract (215 mg/kg)	*C. procera* extract (323 mg/kg)	*C. procera* extract (431 mg/kg)
WBC (10^3/µL)	1.36 ±0.11^{a}	4.57±1.00^{b}	3.19 ±0.78^{ab}	2.82±0.64^{ab}	2.81±0.18^{ab}
Lym (10^3/µL)	1.07±0.08^{ab}	3.74±0.72^{c}	2.34±0.56^{b}	0.89±0.12^{a}	0.96±0.08^{a}
Gran (10^3/µL)	0.18±0.04^{a}	0.25±0.07^{ab}	0.55±0.16^{ab}	1.08±0.39^{ab}	1.15±0.42^{b}
RBC (10^6/µL)	6.29±0.45^{a}	6.18±0.42^{a}	7.82±0.32^{b}	8.64±0.25^{bc}	9.18±0.33^{c}
HGB (g/ml)	12.83±0.81^{a}	12.93±0.33^{a}	12.67±0.23^{a}	14.27±0.23^{a}	14.60±0.95^{a}
HCT (%)	49.73±6.30^{a}	1.23±0.07^{a}	1.53±0.03^{a}	1.57±0.03^{a}	1.63±0.03^{a}
MCV (fL)	78.33±4.72^{a}	69.28±1.44^{a}	65.37 ±7.66^{a}	76.07±1.73^{a}	76.07±6.03^{a}
MCH (Pg)	20.40±0.15^{a}	18.70±0.85^{a}	21.53±2.05^{a}	20.90±2.42^{a}	20.57±2.57^{a}
MCHC (g/dL)	26.30±1.84^{a}	26.97±1.11^{a}	31.03±7.20^{a}	27.77±3.78^{a}	28.10±3.60^{a}
PLT (10^3/µL)	493.33±35.22^{a}	999.67±213.29^{b}	111.90±65.56^{a}	276.33±90.67^{a}	277.67±91.335^{a}
PCT (%)	0.59±0.15^{a}	0.85±0.24^{a}	0.33±0.07^{a}	0.39±0.17^{a}	0.39±0.17^{a}

Values are presented as mean ± SEM (n = 3). Values having the same superscript are not significantly different at (p>0.05) using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0.

and normal hepatocytes arranged in codes (short arrow). While the group administered extract 215 mg/kg (Plate 3) showed congested central vein (long arrow), and normal hepatocytes (short arrow). Group administered extract 323 mg/kg (Plate 4) showed hypertrophied central vein, (long arrow) and mild inflammation of hepatocytes (short arrow). Similarly, the group administered extract 431 mg/kg (Plate 5) showed inflamed hepatocytes (short arrow) and hypertrophied central vein (long arrow).

The subchronic effect of *C. procera* methanol leaf extract on kidney tissues in normal control showed regular glomerulus and normal renal tubules (Plate 6). While the group administered 108 mg/kg revealed regular glomerulus and normal renal tubules (Plate 7). Also, groups administered 215 mg/kg and 323 mg/kg showed regular glomerulus and normal renal tubules (Plates 8 and 9). However, the kidney tissue of group administered extract 431 mg/kg revealed mild inflammation of the glomerulus and normal renal tubules (Plate 10).

In vivo antioxidant potentials of *C. procera* methanol leaf extract

The effect of *C. procera* methanol leaves extract on antioxidant parameters revealed a significant ($p<0.05$) increase in SOD of normal control compared to induced control. Similarly, all extract treated groups and drug control significantly ($p<0.05$) decreased compared to normal control but significantly ($p<0.05$) increased compared to induced control. A significant ($p<0.05$) increase in catalase (CAT) was observed in normal control, drug control and all extract treated groups compared to induced control. There was no significant ($p>0.05$) difference in groups treated with 100 mg/kg extract compared to normal control. Similarly, there was no significant ($p>0.05$) difference in groups treated with 200 and 400 mg/kg compared to drug control. Glutathione reductase (GSH) revealed no significant ($p>0.05$) differences between normal control and induced control. While dose dependent significant ($p<0.05$) increases were observed in all extract treated groups compared to both normal and induced control. Malondialdehyde (MDA) revealed non-significant ($p>0.05$) differences across all treated groups compared to both normal and induced controls. AST and ALT revealed significant ($p<0.05$) decreases in all extract treated groups, drug control and normal control compared to induced control, while there was no significant ($p>0.05$) difference in all extract treated groups and drug control compared to normal control in both AST and ALT. A significant ($p<0.05$) decrease in vitamin C and E were observed in induced control compared to normal control, while there were no significant ($p>0.05$) differences in vitamin C of extract treated groups 100 and 200 mg/kg compared to normal control. The serum vitamin E concentration of drug control and all extract treated groups significantly ($p<0.05$) increases compared to induced control, but however significantly ($p<0.05$) decreases compared to normal control (Table 6).

Effect of *Calotropis procera* methanol leaf extract on histology of liver tissue

The effect of *C. procera* methanol leaf extract on the histology of liver tissue revealed that the liver of the normal control group showed a normal central vein and hepatocytes (Plate 11). The drug control group showed a congested central vein and mild inflammation of hepatocytes (Plate 12). The untreated control group revealed a congested central vein, atrophied portal triad and mild inflammation of hepatocytes (Plate 13). The group treated with extract 100 mg/kg section showed a

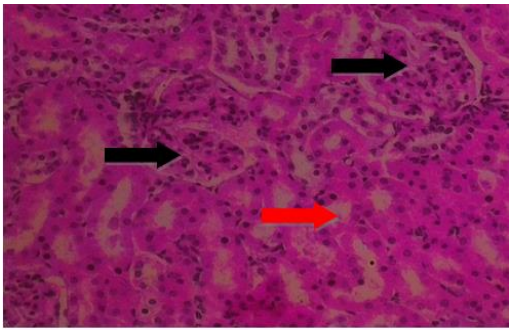

Plate 6. Photomicrograph of rat's kidney obtained from control.

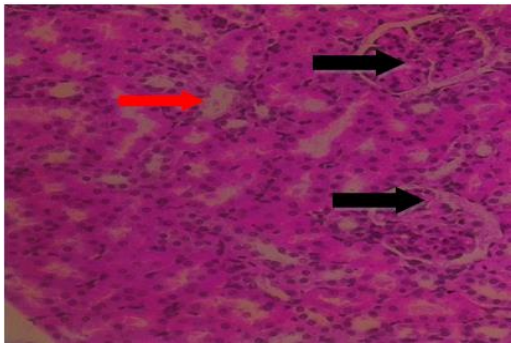

Plate 7. Photomicrograph of rat's kidney obtained from group administered with 108 mg/kg of *C. Procera* methanol leaf extract.

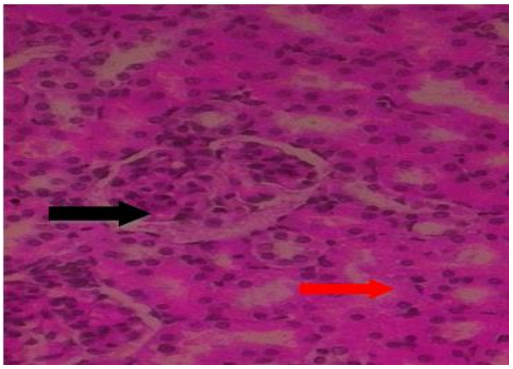

Plate 8. Photomicrograph of rat's kidney obtained from group administered with 215 mg/kg of *C. Procera* methanol leaf extract.

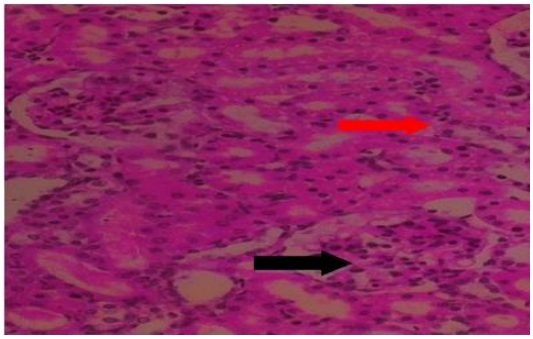

Plate 9. Photomicrograph of rat's kidney obtained from group administered with 323 mg/kg of *C. Procera* methanol leaf extract.

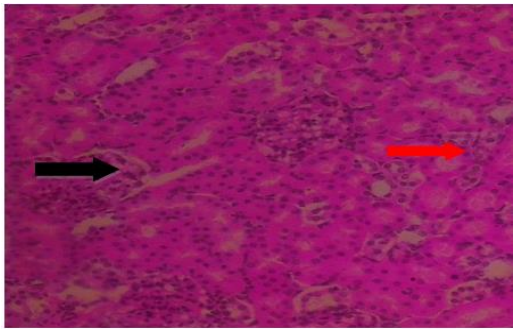

Plate 10. Photomicrograph of rat's kidney obtained from group administered with 431 mg/kg of *C. Procera* methanol leaf extract.

NOTE: Plate 6: Kidney section shows regular glomerulus (black arrows) and renal tubules (red arrow). Plate 7: kidney section shows regular glomerulus (black arrows) and renal tubules (red arrow). Plate 8: Section shows regular glomerulus (black arrow) and renal tubules (red arrow). Plate 9: kidney section shows regular glomerulus (black arrows) and renal tubules (red arrow). Plate 10: kidney section shows mild inflammation of glomerulus (black arrows) and normal renal tubules (red arrow). All tissues were stained with H and E X 100.

shrunken central vein, and hypertrophied hepatocytes (black arrow) (Plate 14). The group treated with extract 200 mg/kg showed mild inflammation of hepatocytes and normal central vein (Plate 15) and the group treated with extract 400 mg/kg section showed normal central vein and mild inflammation of hepatocytes (Plate 16).

Table 6. *In vivo* antioxidant activity of *Calotropis procera* methanol leaf extract.

Dose administered (mg/kg)	SOD (µmole/g)	CAT (µmole/g)	GSH (µmole/g)	MDA (µmole/g)	AST (mg/dl)	ALT (mg/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Normal control (5 mg/kg)	7.45±0.30^{d}	12.57±0.16^{b}	8.13±0.05^{a}	5.24±1.01^{a}	11.33±0.88^{a}	9.00±1.53^{a}	167.22±0.06^{bc}	62.01±0.23^{d}
Standard drug control (0.2 mg/kg)	2.88±0.08^{c}	14.53±0.33^{c}	12.69±0.33^{c}	3.95±0.35^{a}	11.33±0.88^{a}	9.00±1.53^{a}	197.79±6.65^{d}	43.85±3.18^{b}
Induced control (FPN) (10 mL/kg)	1.12±0.06^{a}	7.16±0.57^{a}	7.59±0.69^{a}	11.36±0.66^{b}	31.33±1.45^{c}	25.00±1.15^{b}	106.03±0.27^{a}	39.40±0.29^{a}
CPMLE (100 mg/kg) + FPN	1.81±0.13^{b}	13.08±0.05^{b}	11.02±0.52^{b}	10.80±0.16^{b}	11.00±1.00^{a}	10.67±1.33^{a}	159.15±0.29^{b}	53.12±0.34^{c}
CPMLE (200 mg/kg)+ FPN	2.06±0.02^{b}	14.51±0.18^{c}	12.29±0.21^{c}	8.09±0.02^{a}	11.00±1.00^{a}	9.33±1.33^{a}	171.63±0.25^{c}	54.27±0.33^{c}
CPMLE (400 mg/kg) + FPN	2.70±0.74^{c}	15.08±0.42^{c}	14.00±0.10^{d}	5.26±0.64^{a}	25.33±1.20^{b}	21.33±0.33^{b}	188.51±0.50^{d}	56.07±0.17^{c}

Values are presented as mean ± SEM (n = 5). Values having the same superscript are not significantly different at (p>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. SOD= superoxide dismutase, CAT= catalase, GSH= glutathione reduced and MDA= malondialdehyde.

DISCUSSION

Quite a number of indigenous plants have been reported to contain numerous constituents belonging to different chemical classes of secondary metabolites such as alkaloids, terpenoids, essential oils, glycosides, steroids, phenolic constituents, aliphatic compounds, and polysaccharides. Leaves, stems and roots of the majority of these plants are a rich source of proteins, flavonoids, alkaloids and glycosides (Hussein and El-Anssary, 2019). These active compounds have been exposed to several biological activities, including antiseptic, anti-inflammatory, anti-cancer, antimicrobial and antioxidant activities (Reddy *et al*., 2020). Flavonoids are the most common group of polyphenols that were reported to exhibit various pharmacological properties which include antiviral, anticancer, antioxidant, antihistaminic, anti-inflammatory and hepatoprotective activities (Zahrani *et al.,* 2020). The pharmacological activity observed in the present study might be attributed to the presence of these phytoconstituents.

Additionally, quantitative phytochemical constituents revealed substantial amounts of saponins phenols and flavonoids. Saponin present in all plant species shows that it can be used as lipid lowering agent as well as has anthelmintic and antibacterial activity (Alamgir, 2017). Flavonoids can also inhibit the activity of many enzymes such as xanthine oxidase, peroxidase and nitric oxide synthase, which are supposed to be involved in free radical generation, thereby resulting in decreased oxidative damage of macromolecules (Gwozdzinski *et al*., 2021). The acute toxicity study revealed that *Calotropis procera* has a relatively low LD_{50} of 2154 mg/kg b.wt. The research indicated an increase in body weight of the rats both in the control and treated groups, but the increase in weight is sharper and hence more significant ($p<0.05$) in the group treated with the highest dose. This observation is likely due to the intake of C. procera. It means the group treated with the highest dose of extract gain more weight than the untreated control group due *to C.procera* treatment. This result is in line with that of Zafar *et al.* (2021) which showed a gradual rise in body weight of rats fed with *C. procera* extract. The observed continuous increase in weight of rats in control and test groups has also been reported by Ajagbonna *et al.* (1999). This report, however, is in contrast to the weight loss reported in Wistar rats by Dada *et al.* (2002). Haematology is the specialty responsible for the diagnosis and management of a wide range of benign and malignant disorders of the red and white blood cells, platelets and the coagulation system in animals (Hoffbrand *et al.,* 2019). White blood cells are vital components of the blood. Their role is to fight infection, and they are an essential part of the immune system. A high white blood cell count indicates that the immune system is working to destroy an infection. It may also be a sign of physical or emotional stress (Calder *et al.,* 2020). A mild elevation of monocytes is relatively common and does not usually cause any clinical problems (Riley and Rupert, 2015). Neutrophils are the primary white blood cells that respond to a bacterial infection (Honda *et al.,* 2016). According to Winchester *et al.* (2018), packed cell volume (PCV) and haemoglobin (HGB) are major indices for evaluating circulatory erythrocytes and are very significant in the diagnosis of ailments such as anaemia and also an index for the bone marrow capacity to produce red blood cells in mammals. The results suggested in general that the administration of the aqueous leaf extract of *C. procera* has no toxic effect on the

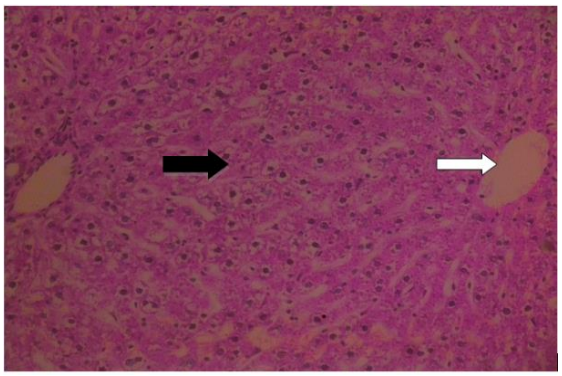

Plate 11. Photomicrograph of rat's liver obtained from control.

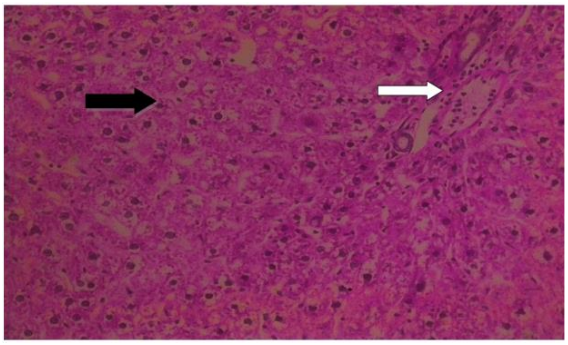

Plate 12. Photomicrograph of rat's liver obtained fr group administered with standard drug 0.2 mg/kg.

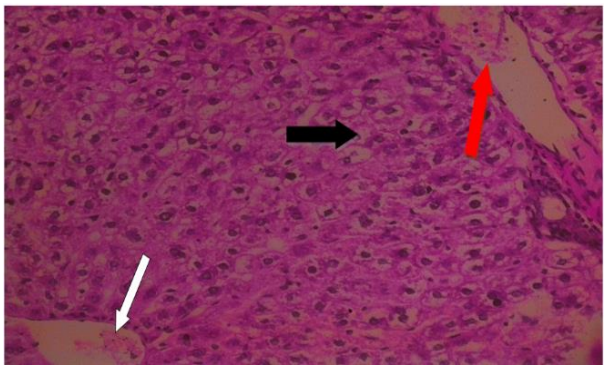

Plate 13. Photomicrograph of rat's liver obtained from untreated (FPN) control.

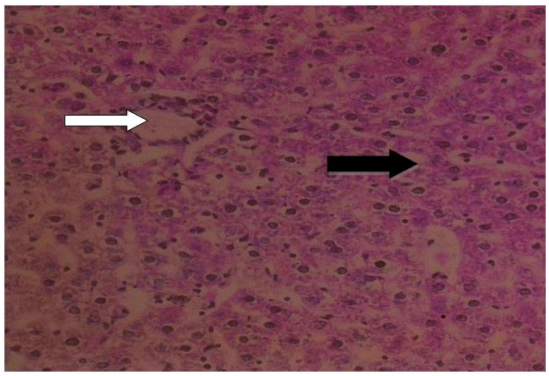

Plate 14. Photomicrograph of rat's liver obtained from group administered with 100 mg/kg of *C. procera* methanol leaf extract.

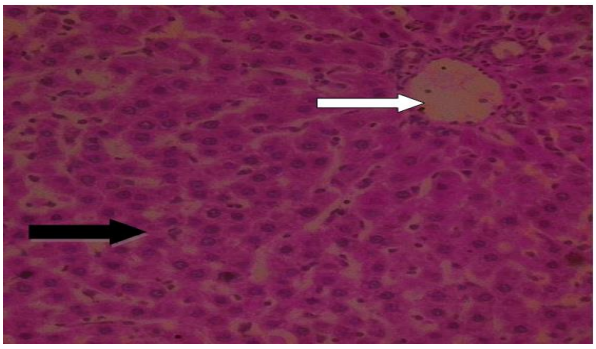

Plate 15. Photomicrograph of rat's liver obtained from group administered with 200 mg/kg of *C. procera* methanol leaf extract.

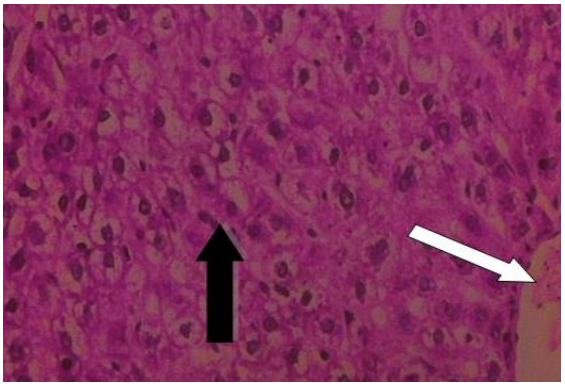

Plate 16. Photomicrograph of rat's liver obtained from group administered with 400 mg/kg of *C. procera* methanol leaf extract.

NOTE: Plate 11: showed normal central vein (white arrow) and normal hepatocytes (black arrow). Plate 12: showed congested central vein (white arrow) and mild inflammation of hepatocytes (black arrow). Plate 13: show congested central vein (white arrow), atrophied portal triad (red arrow) and mild inflammation of hepatocytes (black arrow). Plate 14: Section showed shrunken central vein, (white arrow), cytoplasmic helos and apoptosis of hepatocytes (black arrow). Plate 15: showed mild inflammation of hepatocytes (black arrow) and normal central vein (white arrow). Plate 16: Section showed normal central vein (white arrow) and mild inflammation of hepatocytes (black arrow). All Plates are stained with H and E X 100 magnification.

haematological parameters of the rats when used within a safe dose. These findings about the haematolo-gical parameters are consistent with the results reported by Dada *et al*. (2002) and Mbako *et al*. (2009). Their results indicated an increase in white and red blood cells. Determination of AST and ALT enzyme levels in blood is very significant in order to detect liver damage. If the levels of these enzymes are increased in the blood, that is indicative of liver damage. Results from the renal function analysis revealed an increase in serum creatinine levels in fipronil treated group which reduced significantly in the treated groups. This is a sign of kidney dysfunction in the fipronil treated group. An increase in urea levels was observed in fipronil treated group compared to extract treated recovery groups which also showed a general increase compared to the positive control group. In general, an increase in urea levels is likely due to over production or the inability to excrete it. Significant increases in bilirubin levels were observed in fipronil treated group when compared with the positive control and recovery groups. Elevated bilirubin levels as observed in the fipronil group may indicate an increased rate of destruction of red blood cells (Miao *et al.,* 2019). The toxicity result for AST, ALT and ALP showed no significant increase in the treated groups. During the antioxidant assay, no mortality was recorded in fipronil treated rats at 10 ml/L. From the results of the antioxidant study, it was observed that liver damage was evoked by Fipronil. This is proven by an increase in liver function enzyme values for AST, ALT, ALP and TP when compared to the control and *C. procera* treated groups. AST and ALT are essential in the biosynthesis and catabolism of amino acids, good at neutralising energetic macromolecules (Seven, 2004) and widely used as markers for hepatic damage. SOD and CAT levels were significantly increased. This lends credence to the antioxidant potential of the extract as SOD mops up excess oxygen radicals. However, there was a significant surge in levels of these enzymes at the 323 mg/kg dose. Total protein and albumin showed a significant decrease in the treated groups when compared with the control groups. Though there is a slight dose-dependent rise in protein levels within the treated groups.

The recorded increase in the readings of these enzymes may be due to stress on liver tissue leading to liver membrane permeability. The results revealed that administration of FPN evoked oxidative stress in the hepatic tissues, evident from the generation of lipid peroxidation (LPO). Lipid peroxidation is documented to query the integrity of membranes as a start to various liver ailments (Sharma and Sangha, 2014). Increased malondialdehyde readings in FPN treated group may be due to an increase in the production of reactive oxygen species, such as hydroxyl radicals. From the study, the decrease observed in SOD, CAT and GsH levels could likely be due to the excessive production of oxygen which is quickly converted to H_2O_2 by SOD and then to water by CAT. The presence of higher MDA in the serum is an indication of induced lipid peroxidation and of oxidative stress (Abdelkhalek *et al.,* 2015). In the present study, a high concentration of MDA was observed in induced control animals indicating induced lipid peroxidation and oxidative stress, however, animals treated with extract revealed high degradation of serum MDA. Hence, further supporting the antioxidant activity of *C. Procera* methanol leaf extract.

The negative impact of prolonged acute dosage of *C. procera* methanol extract on the structural and functional integrity of liver and kidney tissues was evidenced by the histopathological findings that highlighted the damage after administration induced inflammation of hepatocytes and hypertrophied central vein as observed in liver tissue. The elevations of serum variables related to kidney function were further supported by histological changes in the kidney as evidenced by mild inflammation of the glomerulus. These histological findings indicate that liver and kidney damage caused by the extract is time-dependent. The results are in contrast to several studies (Kumar and Padhy, 2011; Kumar *et al.,* 2022) that reported hepatoprotective and renoprotective effects of the plant. Such discrepancies may be due to the difference in used doses, plant parts, extract preparation methods and animal species.

Conclusion

This study was conducted to investigate the toxicological and antioxidant effect of *Calotropis procera* leaf methanol extract against fipronil induced oxidative stress and lipid peroxidation in Wistar albino rats. Standard laboratory protocols were employed for the phytochemical screenings, LD_{50} determination, sub-chronic toxicity and *in vivo* antioxidant assay. Based on the result of this research, it can be concluded that *Calotropis procera* leaf extract did not cause much change in the majority of the blood, especially with lower dose administration. The same can be observed in the biochemical and histopathological analyses carried out. It also has a strong antioxidant potential as seen when the control, fipronil and extract treated groups are compared in the anti-oxidant studies. These results show that the administration of *Calotropis procera* methanol leaf extract may be useful in ameliorating the toxic effects of phenyl pyrazole insecticides. This lends credence to its continuous use in folklore medicine, however, it could be relatively toxic if a very high dosage is continuously used over a long period of time.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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