The Journal of Toxicology and Health. Photon 105 (2014) 456-463
https://sites.google.com/site/photonfoundationorganization/home/the-journal-of-toxicology-and-health
Original Research Article. ISJN: 2294-7439: Impact Index: 4.72

The Journal of Toxicology and Health

Photon

Toxicological Evaluation of Methanolic Leaf Extract of *Calotropis procera* (Ait.) R. Br. on Selected Biochemical Parameters in Rats

O.S. Okere[a], M.D. Adams[a]*, S.C. Ifeonu[a], J.O.K. Abioye[b], D.K. Abdullahi[c], O.F. Adebayo[d]

[a] Department of Biochemistry, Bingham University, Karu, Nasarawa State, Nigeria
[b] Department of Biological Sciences, Bingham University, Karu, Nasarawa State, Nigeria
[c] Department of Chemistry, Bingham University, Karu, Nasarawa State, Nigeria
[d] Department of Physiology, Bingham University, Karu, Nasarawa State, Nigeria

Article history:
Received: 25 April, 2014
Accepted: 1 May, 2014
Available online: 07 August, 2014

Keywords:
Calotropis procera, Toxicological evaluation, Asclepiadaceae, Biochemical parameters, Phytochemical screening

Abbreviations:
LD: Lethal dose, AST: Aspartate aminotransferase, ALT: Alanine Aminotransferase

Corresponding Author:
Adams M.D.
M.Sc. Degree holder
Email: mdadamsonline@yahoo.com

Okere O.S.
M.Sc. Degree holder

Ifeonu S.C.
B.Sc. Degree holder

Abioye J.O.K.
PhD Degree holder

Abdullahi D.K.
M.Sc. Degree holder

Adebayo O.F.
M.Sc. Degree holder

Abstract

The methanolic leaf extract of *Calotropis procera* was investigated for its toxicological effect on selected biochemical parameters in rats. Forty five albino rats of both sexes were used for the research work. For the acute toxicity study (LD_{50}), fifteen animals were completely randomized into three groups (A-C) comprising 5 animals each. Animals in group A were administered 1 ml of 500 mg/kg body weight of the extract while those in groups B and C received 1000 and 1500 mg/kg body weight of the extract respectively. Signs of toxicity and number of death were observed and recorded for 14 days. In the experimental design, twenty five fresh animals were completely randomized into five groups (A-E) comprising 5 animals each. Animals in group A (control) were administered 1 ml of distilled water while those in groups B, C, D and E were respectively administered 100, 300, 600 and 1000 mg/kg body weight of the methanolic leaf extract of *C. procera* once daily for 7 days. Preliminary phytochemical screening revealed that the methanolic leaf extract of *C. procera* contains alkaloids, flavonoids, tannins, saponins, steroids, phlobatannins and cardiac glycosides. LD_{50} of the extract was found to be safe up to 1500 mg/kg body weight. When compared with the distilled water treated control animals, the methanolic leaf extract of *C. procera* at all doses significantly ($p<0.05$) increased the concentrations of both total and conjugated bilirubin in the serum of the animals in a dose related manner. Treatment with the extract at all doses significantly ($p<0.05$) increased the activity of AST in the liver, kidney and serum of the animals in a dose related manner. Administration of the extract at all doses significantly ($p<0.05$) increased the activity of ALT in the liver and serum of the animals also in a dose related manner. The extract at all doses significantly ($p<0.05$) decreased the activity of ALT in the heart of the animals. Overall, the methanolic leaf extract of *C. procera* which caused alterations in the biomolecules may affect the normal functioning capacity of the tissues; therefore, it may not be completely 'safe' as oral remedy at the doses investigated in this study.

Citation:
Adams M.D., Okere O.S., Ifeonu S.C., Abioye J.O.K., Abdullahi D.K., Adebayo O.F., 2014. Toxicological Evaluation of Methanolic Leaf Extract of *Calotropis procera* (Ait.) R. Br. on Selected Biochemical Parameters in Rats. The Journal of Toxicology and Health. Photon 105, 456-463.

Photon Ignitor: ISJN22947439D696407082014

1. Introduction

The practice of traditional medicine involving the use of medicinal plant is as old as the origin of man. Two-third of the world population (mainly in the developing countries)

rely entirely on such traditional medical therapies as their primary form of health care without recourse to its possible toxicological implications (Sumana and Suryawanshi, 2001; Saad *et al*., 2006). Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties (Ogunlesi *et al*., 2008). Few plants or their phytochemical constituents have been proven to have medicinal effects by rigorous scientific investigation or have been approved by regulatory agencies such as the United States Food and Drug Administration or European Food Safety Authority (Kumar and Arya, 2006). Historically, the medicinal value of plants was tested by trial and error using the guiding principle of 'Doctrine of Signature'. Doctrine of Signature states that herbs that resemble various parts of the body can be used to treat ailments of that part of the body. Modern approaches to determining the medicinal properties of plants involve collaborative efforts that can include ethnobotanists, pharmaceutical chemists, and physicians (Smith, 2003).

Despite the wide acceptance and consumption of herbal concoction from these medicinal plants, there is little evidence to scientifically verify the shortcomings of their usage or knowing the possible adverse effect they pose. One of such medicinal plants is *Calotropis procera*.

Calotropis procera (Ait.) R. Br. (Family-Asclepiadaceae) is commonly called "Apple of Sodom" in English. It is otherwise known locally in Nigeria as "tufatia" in Hausa, northern Nigeria; "kausa" in Igbo, southeastern Nigeria; "bomu-bomu" in Yoruba, southwestern Nigeria. It is also widely known in French as "pomme de sodome" and in India as "madar or akanal". *Calotropis procera* is a large bushy shrub with decussate, obovate, coriaceous, auriculate, leaves with acute, subsessile apices extra-auxillary, umbellate, panicale inflorescence with purple corolla and erect lobes (Rastogi and Mehrotra, 1999; Nandkarni, 2000). Morphological characteristics revealed the leaves of *Calotropis* to be subsessile, 6-15 cm by 4.5-8 cm, broadly ovate, ovate-oblong, elliptical, acute, pubescent when young and glabrous on both sides on maturity (Lala, 1993; Evans, 2005). The plant is widely distributed from Algeria to Egypt, Nigeria, tropical Africa, France, Warizistan, Afghanistan and India. In Nigeria, it is found in the semi-arid regions of Bauchi, Borno, Kano, Kaduna and most parts of Northern Nigeria (Howard, 1989; Liogier, 1995; Sharma *et al*., 1997; Ahmed *et al*., 2005).

Previous studies by Murti *et al* (2010) have reported the pharmacognostic standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae) while Aradhana *et al* (2011) worked on the toxicological evaluation of *Calotropis procera* against freshwater snails. Mainasara *et al* (2012) have also reported the phytochemical and antibacterial properties of root and leaf extracts of *Calotropis procera* while Erdmann (1983) identified the nutrient and cardenolide composition of unextracted and solvent-extracted *Calotropis procera*. Furthermore, Bharti *et al* (2010) have reported the protective effect of *Calotropis procera* latex extracts on experimentally induced gastric ulcers in rats while Magalhes *et al* (2010) worked on the *in vitro* and *in vivo* antiproliferative activity of *Calotropis procera* stem extract. Moreso, Kareem *et al* (2008) worked on the antimicrobial activities of *Calotropis procera* on selected pathogenic microorganisms while Roy *et al* (2005) studied the antioxidant and protective effect of latex of *Calotropis procera* against alloxan-induced diabetes in rats. Kumar *et al* (2001) worked on the antidiarrhoeal activity of the latex of *Calotropis procera*. The leaves of *Calotropis procera* are said to be valuable as an antidote for snake bite, sinus fistula, rheumatism, mumps, burn injuries, skin disease and body pain (Raghubir *et al*., 1999; Alikhan and Khanum, 2005). The leaves of *Calotropis procera* are also used to treat jaundice (Alikhan and Khanum, 2005). Again, Padhy *et al* (2007) opined that *Calotropis procera* latex affords protection against carbon tetrachloride induced hepatotoxicity in rats while Basu *et al* (1997) reported the hepatoprotectant effects of *Calotropis procera* root extract on experimental liver damage in animals. With all these, there is still dearth of information on the toxicity of its methanolic leaf extract. Therefore, the present research work was designed to evaluate the toxicological effect of methanolic leaf extract of *Calotropis procera* (Ait.) R. Br. on selected biochemical parameters using rats as model.

2. Objective of Research

The main objective of this study is to evaluate the toxicological effect of methanolic leaf extract of *Calotropis procera* (Ait.) R. Br. on selected biochemical parameters in rats.

3. Justification of Research

The methanolic leaf extract of *Calotropis procera* has been used in the management of many ailments without recourse to its toxicological implications. Also, there is paucity of information on the toxicity of this medicinal plant in open scientific literature. Therefore, the present research work was designed to evaluate the toxicological effect of methanolic leaf extract of *Calotropis procera* (Ait.) R. Br. on selected biochemical parameters using rats as model.

4. Materials and Methods

4.1 Materials

4.1.1 Plant Materials and Authentication

Fresh leaves of *Calotropis procera* was obtained within the premises of Bingham University, Karu, Nigeria and was authenticated with a voucher specimen (NISCAIR/RHMD/Consult/-2008-09/1144/176).

4.1.2 Experimental Animals

Albino rats (*Rattus norvegicus*) of both sexes weighing 120.34± 4.26 g were obtained from the Department of Biochemistry, Animal House, University of Jos, Jos, Nigeria.

4.1.3 Assay Kits and Chemicals

The assay kits for the determination of total and conjugated bilirubin, aspartate aminotransferase and alanine aminotransferase were products of Randox Laboratory Ltd, Co-Atrium, Uk. Sodium hydroxide which was used for the preparation of 0.4 N sodium hydroxide employed as one of the reagents for the determination of aspartate and alanine aminotransferase respectively.

4.1.4 Other Reagents

All other chemicals and reagents used which were of analytical grade were products of sigma Aldrich Ltd., Buchs, Canada and are prepared in volumetric flask using glass wares with distilled water. The reagents were stored in neat, air-tight reagent bottles except for the Biuret reagent which was stored in plastic containers.

4.2 Methods

4.2.1 Preparation of Methanolic Leaf Extract of Calotropis procera

Fresh leaves of *Calotropis procera* were air dried under a shade until a constant weight was obtained. This was thereafter pulverized in a blender (PHILIPS, Model HR-1724, Brazil). A known weight (150 g) of the powder was extracted in 1000 ml of methanol for 72 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper (Maidstone, UK) and the resulting filtrate concentrated in a Rotary Evaporator, where some methanol was recovered. The mixture was further transferred into steam bath where it was evaporated to give the required brownish-black residue. This was then reconstituted in distilled water to give the required doses used in the present study.

4.2.2 Phytochemical Screening

Preliminary phytochemical screening to detect the presence of alkaloids, saponins, tannins, flavonoids, anthraquinones and cardiac glycosides were carried out by adopting the procedures described by Harborne (1973), Walls *et al*., (1996), Odebiyi and Sofowora (1978), Awe and Sodipo (2001), Mainasara *et al*., (2012) and Sofowora (1993) respectively while those of phlobatanins, steroids, and terpenoids were determined by the procedures described by Trease and Evans (1983).

4.2.3 Acute Toxicity Study (LD_{50})

The lethal dose (LD_{50}) of methanolic leaf extract of *Calotropis procera* was carried out by the method described by Lorke (1983). Briefly, fifteen (15) animals were completely randomized into three groups (A-C) comprising 5 animals each. Animals in group A were administered 1 ml of 500 mg/kg body weight of the extract while those in groups B and C received 1000 and 1500 mg/kg body weight of the extract respectively. Signs of toxicity and number of death of animals were observed and recorded for 14 days.

4.2.4 Animal Grouping and Extract Administration

A total of twenty five (25) fresh albino rats, housed in clean aluminum cages contained in well ventilated standard housing conditions (temperature: 28-31° C; photoperiod: 12 hours; humidity: 50-55%) was used for the study. The animals were allowed free access to rat pellets (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and tap water *ad libitum*. The cages were also cleaned on daily basis. The animals were acclimatized for two weeks before the commencement of the experiment. For the experimental design, the albino rats were completely randomized into five groups (A-E) comprising 5 animals each. Animals in group A (control) were administered 1 ml of distilled water while those in groups B, C, D and E were respectively administered 100, 300, 600 and 1000 mg/kg body weight of the methanolic

leaf extract of *C*. *procera*. The administration was done once daily for 7 days using metal oropharyngeal cannula. The animals were handled humanely according to the guidelines of European convention for the protection of vertebrate animals and other scientific purposes- ETS-123 (European Treaty Series, 2005).

4.2.5 Preparation of Serum and Tissue Homogenate

The rats were anaethesized in a glass jar containing cotton wool soaked in diethyl ether. The unconscious rats were quickly removed and the neck area cleared of fur. The jugular vein which was slightly displaced (to avoid contamination of the blood with interstitial fluid) was cut with a sterile scapel blade and an aliquot of the blood was collected into a sample bottle. The blood was then left undisturbed for 10 minutes at room temperature to clot. The blood was thereafter centrifuged at 224x g for 10 minutes using Uniscope Laboratory Centrifuge (Model 800D, New Life Medical Instrument, England). The sera were later aspirated with Pasteur pipette into dry, sample bottles and used within 12 hours of preparation for the biochemical assays. The animals were thereafter quickly dissected and the liver, kidney and heart were removed. The organs were later blotted with clean tissue paper, weighed and homogenized in 0.25 M sucrose solution (1:5 w/v). The homogenates were then centrifuged at 1340 x g for 15 minutes. The supernatant was frozen overnight (Ngaha *et al*., 1989) before being used for the determination of biochemical parameters.

4.2.6 Statistical Analysis

Results were expressed as the mean ± SEM of five determinations. The data were analyzed using Duncan Multiple Range Test and complemented with Student's t-test. The differences were considered statistically significant at $p<0.05$. All these analyses were done using SPSS 20.0 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

5. Results

Preliminary phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, phlobatannins and cardiac glycosides while terpenoids and anthraquinones were not detected (Table 1).

Table 1: Phytochemical Constituents of the Methanolic Leaf Extract of *Calotropis procera*

Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Steroids	+
Terpenoids	-
Anthraquinones	-
Cardiac glycosides	+
Phlobatannins	+

Key: + = Detected
- = Not detected

Table 2: Effect of Methanolic Leaf Extract of *C. procera* on Serum Bilirubin Concentration of Rats

Parameters	methanolic leaf extract of *C. procera* (mg/kg bogy weight)				
	Control	100	300	600	1000
(µmol/L)					
Serum total	52.17±0.18^{a}	90.47±0.37^{b}	65.49±0.18^{c}	59.39±0.18d	58.55±0.27^{d}
Bilirubin					
Serum	66.42±0.18^{a}	103.80±0.18^{b}	73.28±0.18^{c}	70.67±0.18^{c}	54.30±0.27^{d}
Conjugated					
Bilirubin					

Data are mean ± SEM of five determinations. Test values carrying superscripts different from the control for each parameter across the row are significantly different ($p<0.05$)

Table 3: Effect of Methanolic Leaf Extract of *C. procera* on Aspartate Aminotransferase Activity in the Liver, Kidney and Serum of Rats

	Methanolic leaf extract of *C*. *procera* (mg/kg bogy weight)				
Parametes	Control	100	300	600	1000
(U/L)					
Liver AST	24.25±0.14^{a}	27.94±0.33^{b}	30.66±0.19^{c}	31.60±0.31^{c}	34.83±0.14^{d}
Kidney AST	26.00±0.51^{a}	56.68±0.26^{b}	63.10±01.3^{c}	66.86±0.53^{d}	67.10±0.17^{d}
Serum AST	29.03±0.47^{a}	67.71±0.31^{b}	71.41±1.88^{c}	75.96±0.27^{d}	88.93±0.25^{e}

Data are mean ± SEM of five determinations. Test values carrying superscripts different from the control for each parameter across the row are significantly different (*p<0.05*)

Table 4: Effect of Methanolic Leaf Extract of *C. procera* on Alanine Aminotransferase Activity in the Liver, Heart and Serum of Rats

Parameters	Methanolic leaf extract of *C. procera* (mg/kg bogy weight)				
	Control	100	300	600	1000
(U/L)					
Liver ALT	4.44±0.06[a]	65.72±0.17[b]	66.96±0.11[b]	67.66±0.16[b]	70.09±0.27[c]
Heart ALT	104.90±0.11[a]	75.88±0.07[b]	77.28±0.05[c]	78.38±0.22[c]	78.62±0.28[c]
Serum ALT	6.39±0.80[a]	85.19±0.11[b]	89.75±0.05[b]	92.11±0.05[c]	93.44±0.28[c]

Data are mean ± SEM of five determinations. Test values carrying superscripts different from the control for each parameter across the row are significantly different (*p<0.05*)

In the acute toxicity study, all the graded doses of the methanolic leaf extract of *Calotropis procera* administered to the animals showed no signs of toxicity and no deaths were recorded. Therefore, the LD_{50} of methanolic leaf extract of *Calotropis procera* was found to be safe up to 1500 mg/kg body weight.

When compared with the distilled water treated control animals, the methanolic leaf extract of *C*. *procera* at all doses significantly (*p<0.05*) increased the concentrations of both total and conjugated bilirubin in the serum of the animals in a dose related manner (Table 2). Administration of the extract at 300 and 600 mg/kg body weight did not significantly (*p>0.05*) alter the concentration of conjugated bilirubin in the serum of the animals (Table 2).

Administration of the extract at all doses significantly (*p<0.05*) increased the activities of alanine aminotransferase (ALT) in the liver and serum of the animals. The extract at 100, 300 and 600 mg/kg body weight did not significantly (*p>0.05*) alter the activity of ALT in the liver of the animals. Administration of the extract at 100 and 300 mg/kg body weight as well as 600 and 1000 mg/kg body weight did not significantly (*p>0.05*) alter the activity of ALT in the serum of the animals (Table 4). Administration of the extract at all doses significantly (*p<0.05*) decreased the activity of alanine aminotransferase ALT in the heart of the animals. Treatment with the extract at 300, 600 and 1000 mg/kg body weight did not significantly (*p>0.05*) alter the activity of ALT in the heart of the animals (Table 4).

6. Discussion

Although, poisonous plants abound everywhere, herbal medicine is still used by up to 80% of the population of developing countries. Despite widespread use, few scientific studies have been undertaken to ascertain the safety or toxicity risks of many herbal remedies (Klaassen and Eaton, 1991). Bilirubin is an important metabolic product of blood with biological and diagnostic values (Moudgil and Narang, 1989). The increase in total and conjugated bilirubin levels in the serum of the animals by the extract at all doses may be an indication of enhanced functional capacity of the liver as extensive synthesis may lead to increased serum levels of bilirubin (Moudgil and Narang, 1989). This increase is hepatotoxic and might adversely affect the normal functioning capacity of the hepatocytes.

Measurement of the activity of an enzyme is an indispensable tool in the assessment of cellular toxicity caused by chemical compounds including plant extracts (Malomo, 2000; Yakubu *et al.,* 2003) long before structural damage that can be picked by conventional histological techniques (Akanji, 1986). The aminotransferases considered in this study are useful markers of liver cytolysis and can be used in assessing damage in the liver and heart (Chapatwala *et al*., 1982; Shahjahan *et al*., 2004). The aminotransferases are involved in the transfer of amino group from α-amino to α-keto acids and in the biochemical regulation of intracellular amino acid pool. The significant increase in the activities of aspartate aminotransferase (AST) in the liver and kidney of the animals following the administration of methanolic leaf extract of *Calotropis procera* at all doses may be due to increase either from *De novo* synthesis of the enzyme molecule or an adaptation by the organs to the assault from the drug/plant extract leading to activity higher than the control (Yakubu *et al*., 2001). Such an increase in enzyme activity might have resulted from increase in the functional activity of the liver and kidney (Brain and Kay, 1927). The presence of AST in very large quantities in the liver and kidney might affect the transamination reaction and thus

biochemical regulation of intracellular amino acid pool. The increased serum AST activity in the present study may be adduced to an increase in the functional activity of the enzyme in the serum of the animals.

The decrease in the activity of alanine aminotransferase (ALT) in the heart of the animals by the extract at all doses could be attributed to either inhibition of the enzyme activity at the cellular/molecular level (Akanji *et al*., 1993) or inactivation of the enzyme molecule *in situ* (Umezawa and Hooper, 1892). It may also affect other metabolic process where the enzyme is involved; such as synthesis of nuclear proteins, nucleic acids and phospholipids as well as in the cleavage of phosphate esters (Ramalingam and Vimaladevi, 2002). The significant increase in the activity of ALT in the liver and serum following administration of the extract at all doses could be due to *De novo* synthesis of the enzyme molecule leading to high activity in the liver and serum of the animals.

Conclusion

The present study revealed that methanolic leaf extract of *Calotropis procera* did not cause any apparent toxicity to the animals. The study also showed that the extract has adverse effects on the serum bilirubin which would affect liver function of the animals. Alterations in the activity of the enzymes suggest that the extract might interfere with the normal functioning of the enzymes in the liver, kidney and heart of the animals. Therefore, the methanolic leaf extract of *C. procera* may not be completely 'safe' as oral remedy at the doses investigated in this study.

Limitations

The present study only considered the toxicological implications of methanolic leaf extract of *Calotropis procera* on selected biochemical parameters of bilirubin, AST and ALT in the serum, liver, kidney and heart of the animals. It also screened the plant for its phytoconstituents as well as its lethal dose (LD_{50}). The study could not characterize the extract using gas chromatography/mass spectrometry (GC/MS) or High Performance Liquid Chromatography (HPLC). Extract administration was for seven days. Histology of tissues was not carried out.

Recommendations

For further studies, the crude extract should be isolated, characterized and purified to be sure of the actual bioactive agents. The number of days for extract administration should also be increased since the seven days of extract administration might not be sufficient enough to reveal profound functional toxicity. Further studies should carry out histology of tissues which would provide information on whether the extract has the structural toxicity on the tissues or not. This shows that further studies are required, which would be long-term, and multiple tissues would be involved to examine the effect of dose and duration of administration of methanolic leaf extract of *C. procera* on the biological system.

Funding and Policy Aspects

We recommend that research grant should be given to researchers to improve on existing findings in science and technology. We declare that this study was not financially supported by any research institute, but by the authors.

Acknowledgement

We acknowledge the contributions of Department of Biochemistry, Bingham University, Karu, Nasarawa state, Nigeria for their assistance.

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