



TOXICITY EVALUATION OF ALKALOIDS FROM *Calotropis procera* LEAVES USING *Drosophila melanogaster*

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ABSTRACT

Calotropis procera is a xerophytic perennial shrub native to tropical and subtropical Africa, Asia and common in the Middle East, belonging to the family Asclepiadaceae. Alkaloids are a group of naturally occurring organic compounds that mostly contain nitrogen atoms in a heterocyclic ring produced by a large variety of organisms. The study aims to evaluate the toxicity of alkaloids extracted from the leaves of *Calotropis procera* plant. The maceration extraction method was employed to extract alkaloid compounds from the leaves of the plant and phytochemical screening was conducted to confirm that the extract contains alkaloids. *Drosophila melanogaster* (fruit flies) of 3-4 days old were used for toxicity studies of the alkaloid extract and the result showed that at concentrations of 62.5 mg – 250 mg, 100 % mortality occurred while at concentrations of 1.25 mg – 5 mg with sub-acute toxicity, and 12.5 mg, 25 mg and the control group with low percentage mortality. In conclusion, alkaloids from *Calotropis procera* leaves were found to be toxic to *D. melanogaster*.

Keywords: Alkaloids, *Calotropis procera*, *Drosophila melanogaster*, maceration, toxicity

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INTRODUCTION

Most plants contain phytochemicals that are toxic and can serve as defense against various diseases caused by bacteria, viruses and fungi as well as protectors from herbivores [1]. These compounds also known as phytotoxins are poisonous substances produced by plants or plant pathogens that have adverse effects on living organisms. Toxins can be tiny molecules, peptides, or proteins that are capable of causing disease on contact with or absorbed by body tissues, interacting with biological macromolecules such as enzymes or cellular receptors. They have a great diversity in their composition, occurrence, mode of action and lethal

effects [2]. Phytotoxins are commonly classified and described according to their source, diversified structure, chemistry, clinical relevance, toxicity mechanisms and health effects [3].

Calotropis procera is a member of the Asclepiadaceae subfamily with over 180 genera and 2200 species distributed in almost all tropical and subtropical regions of the world. The plant has an economic importance and is found to have an attractive medicinal function in traditional medicine. The plant secretes milky latex that is present in all its tissues and is traditionally used as a purgative; its flowers are used to treat a variety of conditions, such as respiratory problems like cough and asthma, as well as appetite loss; and they are used as stomachic

and digestive tonics because of their anti-inflammatory, expectorant and carminative qualities. In addition to promoting secretion, the root bark is used to treat intestinal parasites, ascites, skin conditions, abdominal organ enlargement and generalized body swelling (anasarca) [4].

Calotropis belongs to the Apocynaceae family, which is commonly known as milkweed or Aak or Sodom apple. Plants of this family are referred to as milkweed because they produce a white and sticky latex from different parts of the plant. The plant has two common species *Calotropis procera* and *Calotropis gigantea* which are described based on the pharmacological properties [5].

A study on the effects of aqueous extract of *Calotropis procera* leaves in sub chronic poisoning of goat by [6], stated that phytochemical screening of *C. procera* leaves shows the presence of cardiac glycosides, alkaloids, terpenoids, saponins, phenols, tannins and flavonoids as the bioactive compounds and have been responsible for the medicinal and toxicological characteristics.

Alkaloids are a class of naturally occurring chemical compounds that contain carbon, hydrogen, nitrogen and oxygen atoms. They are produced by a large variety of living organisms like animals, bacteria, fungi and plants. It is among the diverse class of secondary metabolites which play a vital role in the ecology of organisms and are synthesized to act as a defence mechanism against pathogens and animals [7, 8]. Most natural alkaloids possess anti-amyloid, anti-inflammatory and antioxidant features as well as anti-depressive and anti-convulsing effects. Some alkaloids have been known to have toxicity characteristics, although almost each of the alkaloids has its adverse effects [8]. Apart from their general name (alkaloids) they have many varieties of structures, some of which have been known for a

long because of their wide range of usage on human and other animal species.

Alkaloids from *Calotropis procera* are very poisonous, with specific effects on eye health and general physiological processes. Studies show that these harmful effects, which can cause disorders like keratoconjunctivitis and corneal edema, which can impede vision without causing discomfort, are mostly caused by alkaloids, especially calotoxin [9].

Drosophila melanogaster (fruit fly); is a dynamic model animal in many biological research domains because of its quick life cycle, genetic simplicity, and accessibility to cutting-edge genetic resources. Its uses include everything from researching disease mechanisms to finding new drugs and conducting ecological studies. *Drosophila* has played a significant role in helping us understand fundamental genetic principles such as sex-linked inheritance and mutation mechanisms and because of its similar brain structure with that of humans, it is also utilized to research neurodegenerative disorders, including as Parkinson's and Alzheimer's [10]. The fruit fly is being employed more and more in drug development, especially in the study of cancer, and has produced promising customized therapy alternatives and enables quick evaluation of possible medications [11, 12]. Additionally, *Drosophila* models for a variety of malignancies have been created, demonstrating the critical signaling pathways that humans and flies share [12].

Drosophila is also a useful model for researching pesticide resistance. Through comprehending the genetic processes underlying resistance, researchers can create more potent pest management plans. This study aims to evaluate the toxicity of alkaloids extracted from the leaves of *Calotropis procera* using *Drosophila melanogaster*.

MATERIALS AND METHODS

Sample collection, identification and preparation

Fresh leaves of *Calotropis procera* were obtained from the Ribadu Campus of Nigerian Defence Academy in Kaduna State. The plant leaves were taken to the Botanical Section of the Department of Biological Science, Faculty of Science, Nigerian Defence Academy for identification, authentication and NDA/BIOH/2023/11 was obtained as voucher number of the plant samples. The leaves were washed twice with distilled water to remove dirt and filthy particles from the surface of the leaves. The cleaned plant leaves were air dried under the shade for 14 days and were then pulverized into fine powder using the *Herzog vibration grinding mill* HP-M100P.

Maceration method of extraction

Maceration method of extraction was used to carry out the extraction of alkaloids from *Calotropis procera* using the method adopted by Djilani *et al.* [13] with a slight modification, 50 g of the powdered plant material was weighed and wetted with 100 mL of 25% NH₄OH and 200 ml of ethyl acetate was used to carry out extraction at room temperature for 72 hours. The solvent was filtered with muslin cloth then with Whatmann no. 1 filter paper and evaporated using a rotary evaporator under reduced pressure at 40°C. The residue was dissolved in 50 mL of 2% H₂SO₄, then was extracted with 3 × 20 mL petroleum ether to remove acidic, lipophilic and neutral material. The aqueous solution was basified with 25% NH₄OH to pH 8 - 9 and extracted with 3 × 20 ml chloroform. The chloroform extract was dried with Na₂SO₄ and was also concentrated to dryness under reduced pressure using a rotary evaporator to obtain crude alkaloids.

Phytochemical screening of the alkaloid extract

Mayer's test was used to confirm the extract as alkaloid extract. To 2 mg of the extract, 2 drops of Mayer's reagent were added, and a white precipitate showed a positive result for alkaloids [14].

Dragendorff's test was also used to test for the presence of alkaloids in the extract. 2 mg of the extract was acidified with 2M HCl, and 1 mL of Dragendorff's reagent was added. Formation of orange or red precipitate confirmed that the extract contains alkaloids [15].

Drosophila melanogaster stock and culture

Wild-type *Drosophila melanogaster* (Harwich strain) – fruit flies were obtained from the National Species Stock Centre (Bowling Green, OH, USA). The flies were bred and nurtured on cornmeal medium containing brewer's yeast (1% w/v), agar-agar (1% w/v), and nipagen (preservative, 0.08% v/w) at constant temperature (24 ± 2°C) and 70% humidity under 12 h dark/light cycle in the *Drosophila* Laboratory, Department of Biochemistry, Faculty of Physical Sciences, Kaduna State University, Kaduna State Nigeria [16].

Toxicity studies (LC₅₀) and incapacitation effect test of the alkaloid extract on *Drosophila melanogaster*

mortality study (LC₅₀)

The toxicity study was evaluated as described according to Iorjiim *et al.* [17] method with slight modifications. The fruit flies were divided into nine (9) different groups with each group receiving different concentration of the Alkaloid extract from small to medium to high concentrations (i.e. 1.25 mg, 2.5 mg, 5 mg, 12.5 mg, 25 mg, 62.5 mg, 125 mg, 187.5 mg and 250 mg) were incorporated into 2.5 g

each of the flies' diet and then transferred into empty sterilized treatment vials, with group 1 containing only 2.5 g of the diet as normal control (diet only) and were prepared in replicates of three (3) vials per group, with each vial containing twenty (20) fruit flies of both sexes (males and females). *Drosophila melanogaster* flies were transferred into the vials containing the diet and Alkaloid extract. On day three (3), the flies were transferred into a newly prepared diet and Alkaloid extract of the same concentrations. Mortality was recorded after every 24 hours for seven (7) days.

Statistical analysis

The data obtained were expressed as mean \pm standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to analyze the data, followed by Tukey's HSD post hoc test to identify statistically different test groups. XLSTAT statistical software version 2019.2 was used. Statistically significant results were considered at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1: Showing the results for alkaloids extracted from leaves of *Calotropis procera* plant sample and the methods employed

Sample location	Extraction method	Result
Kaduna	Maceration	+

Table 2: Percentage yield of alkaloid extract from *Calotropis procera* leaves

Solvent	Yield (g)	Yield (%)
Chloroform	2.583	2.6

Table 3: Phytochemical screening of the alkaloid extract

Test	Result
Mayer's	+
Dragendorff's	+

Table 2, shows the quantity of alkaloid compounds obtained when 50 g of the plant leaves sample was immersed into the solvent was 2.583 g. This was weighed with analytical balance after drying with Na_2SO_4 .

Table 3 presents the results obtained from phytochemical screening of the alkaloid extract using Mayer's and Dragendorff's tests. This finding is in line with the result of [18] He used several methods to confirm the presence of phytochemicals in a methanolic extract of *Calotropis procera* leaves where Wagner's test was used to confirm the presence of alkaloids in the extract of the leaves of the plant. Also, a study on the hydroalcoholic extract of *Calotropis procera* leaves by [19] have yielded many bioactive compounds with composition and anti-inflammatory activity. The plant leaves extract was found to have numerous phytochemicals including alkaloids, glycosides, sugar, tannins, flavonoids and steroids.

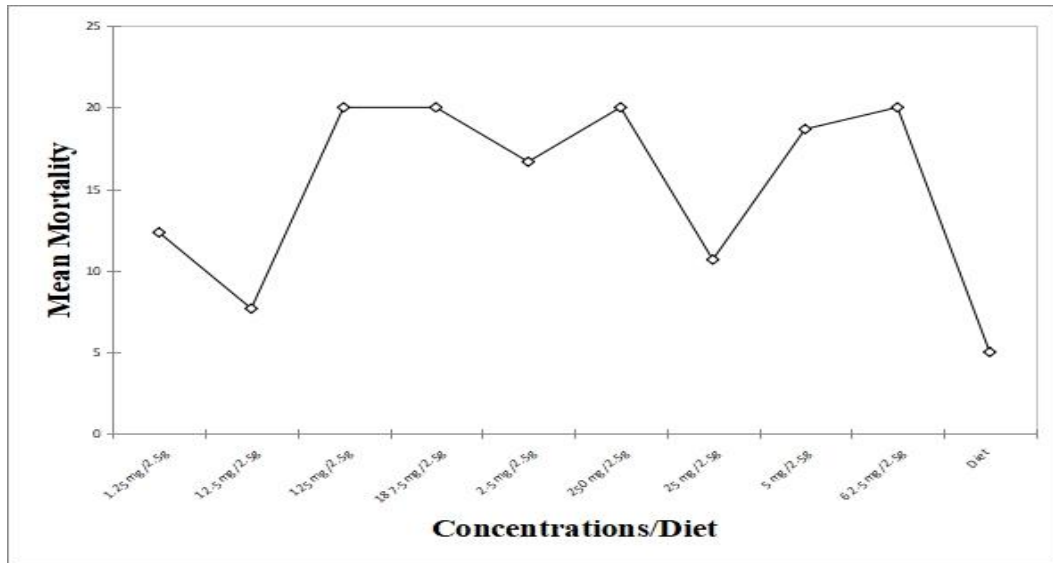


Figure 1: The average mortality of fruit flies in each group

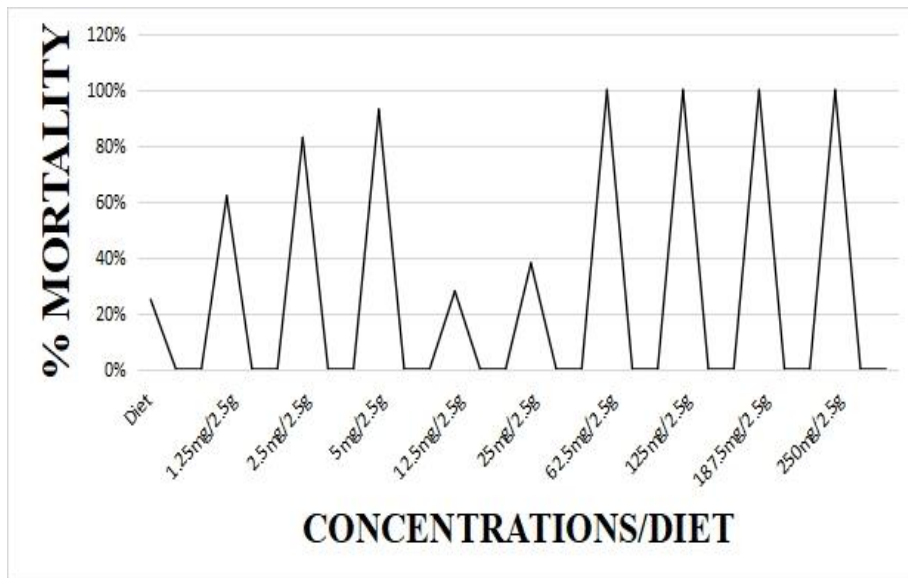


Figure 2: The percentage mortality of fruit flies in various groups

In this study, *Drosophila melanogaster* flies were fed different concentrations of alkaloid extract incorporated into their diet to verify its toxicity in terms of mortality.

From figure 2, at the high concentration of 12.5 mg/2.5g, 125 mg/2.5g, 187.5 mg/2.5g and 250 mg/2.5g there was 100 % death of the flies within the first three (3) days (72 hours). The alkaloid extract showed acute dose-dependent toxicity of LC₅₀ at low concentrations of 63 % mortality at 1.25 mg/2.5g, 83% mortality at 2.5 mg /2.5g and 93% mortality at 5 mg/2.5g respectively. There were no statistically significant differences (P<0.05) between the control (diet only) group and the other concentrations mentioned above; and there was no significant difference between the control group and 12.5 mg, 25 mg concentrations in terms of mortality.

Alkaloids are categorized as naturally occurring compounds comprising carbon, hydrogen, nitrogen, and oxygen, primarily present in flowering plants [8]. Certain alkaloids identified in plants, such as tropane, hyoscyamine, hyoscyne, atropine and scopolamine, exhibit toxicity, particularly upon high consumption [20]. *Drosophila melanogaster* is highly regarded as a model organism for exploring the diverse toxicities of chemical compounds due to its cost-effectiveness, ease of maintenance, high reproductive capacity and genetic similarities to humans [21].

The experimental flies solely consumed their regular diet, supplemented with alkaloid extract at varying concentrations. Findings demonstrated that the alkaloid extract derived from *Calotropis procera* leaves induced 100% mortality in the flies at medium concentrations of 62.5 mg to high concentrations of 125 mg, 187.5 mg, and 250 mg. The toxicity of the alkaloid extract LC₅₀ occurred at low concentrations of 1.25 mg, 2.5 mg, and 5 mg where more than 50% of *D. melanogaster* died, with mortality rates ranging from 63% to 93% [22]. This indicates the toxic nature of these alkaloid extracts at high concentrations, aligning with the research of [23, 24, 25] who reported that alkaloid toxicity is dependent on factors such as dosage, exposure duration, individual characteristics like sensitivity, site of action, and developmental stage. Furthermore, [20] have documented the toxicity of the *Calotropis procera* plant, cautioning against its ingestion by grazing animals due to its toxic properties; historically, the latex from this plant has been utilized as a toxin in arrow poisons.

CONCLUSION

Calotropis procera plant has been commonly used in herbal medicine for the treatment of many diseases and some researchers reported that its latex was also used as poison in warfare. The plant is usually very toxic to grazing animals when consumed, possibly due to alkaloids in the plant leaves. Alkaloid

compounds were extracted from the leaves of *Calotropis procera*, phytochemically screened and quantified. In this study, the mortality of *Drosophila melanogaster* (fruit flies) was recorded after exposure to different concentrations of alkaloid extract incorporated into their diets. The high mortality of the flies could be due to the toxicity of the alkaloids of *Calotropis procera*. Therefore, the alkaloid compounds extracted from the leaves of *Calotropis procera* were found to be toxic to the fruit fly.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of *Drosophila* Laboratory staff, Department of Biochemistry, Kaduna State University, Kaduna, Nigeria. The assistance of the Chief Technologist, Department of Biochemistry, Kaduna State University, Kaduna, Nigeria is highly appreciated.

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