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TOXICITY EVALUATION OF *CEIBA PENTANDRA* (L) GAERTN ACETONE LEAF EXTRACT USING *DROSOPHILA MELANOGASTER* MODEL

Joy J. Adeyemi*[1,2], John O. Ayorinde[1], Amos O. Abolaji[2]

[1]Department of Pharmaceutics, University of Ibadan, Ibadan, Nigeria.
[2]Drosophila Research Laboratory, Department of Biochemistry, University of Ibadan, Ibadan, Nigeria.

***Corresponding Author: Joy J. Adeyemi**

Department of Pharmaceutics, University of Ibadan, Ibadan, Nigeria.

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ABSTRACT

Toxicity studies are used to determine the safety of materials with pharmacological actions when used in animal models. It also establishes the safe limits in conditions of use. There have been previous reports as regards the safety of *Ceiba pentandra* (C. pentandra) extracts in mammalian models which have been reported to be up 5000 mg/ 5g diet, however, its acetone extract has never been reported. Further, the use of *Drosophila melanogaster* models has not been used to determine the plant's safety limits. This study, therefore is aimed at evaluating the acute toxicity of the acetone extract of *C. pentandra* leaves, using *Drosophilia melanogaster* models. Mucilage was obtained by soaking the dried leaves in acetone for two days. The phytochemical screening of the extract was carried out. The acetone extract was administered to *Drosophila melanogaster* in various doses (1mg, 1.5mg, 2mg and 2.5 mg)/ 5g diet and at higher concentrations. There was improvement of survival of *Drosophilia melanogaster* at low concentrations of the extracts whereas at higher concentrations exceeding 75mg/ 5g diet their survival rate reduced. It can be concluded from this study that it is relatively safe at lower dietary levels while exhibiting a dose-dependent toxicity.

KEYWORDS: Toxicity studies; Pharmacological actions; *Ceiba pentandra; Drosophila melanogaster;* mucilage.

INTRODUCTION

Toxicity studies can be defined as a set of systematic investigations used to assess the harmful effects of drugs and chemicals on living organisms, especially animals. It is crucial in drug development and ensures that potential medicinal products do not pose significant risks to human health. These tests can be categorized based on duration and dosing frequency, into acute, sub-acute, sub-chronic, and chronic toxicity studies.

- Acute Toxicity Studies assess the immediate effects of a single dose, often measured by the median lethal dose (LD_{50})[1]
- Sub-acute and Sub-chronic Studies evaluate long-term effects focusing on repeated dosing and its impact on various biological systems.[2]
- Chronic Toxicity Studies investigate prolonged exposure effects, typically lasting a significant portion of the animal's lifespan.[3]

Toxicity studies are very important in drug development being integral to preclinical safety assessments. It helps to prevent the clinical use of harmful substances and identify adverse effects while also guiding the safe dosage for human trials.[4] Findings obtained from these studies inform regulatory decisions and contribute to the safety profile of new compounds.[1]

Animals became standardized for toxicity testing in the early 20th century and have been used since.[5] However, contemporary practices are increasingly scrutinized, which has resulted in the development of ethical frameworks aimed at reducing animal use in research. Therefore, there is a growing shift towards alternative methods in order to reduce reliance on animal testing.[6]

Drosophila melanogaster, commonly known as the fruit fly, is used in this research because it is a widely used

model organism in biological and medical research because of its numerous advantages over traditional animal models like mice and rats. The advantages of using Drosophila over other animal models are manifold because it shares a significant portion of its genetic makeup with humans, with about 60% of human disease-causing genes having recognizable counterparts in the fruit fly allowing researchers to study human diseases effectively.[7] Secondly, is its cost-effectiveness and ease of maintenance and also its short life cycle and high reproductive rate which enable rapid generation turnover, facilitating quick experimental results.[8,9]

C. pentandra (L.) Gaertn (family Malvaceae*)* is a plant that has a wide distribution in equatorial Africa and naturalized humid areas[10] and commonly known as silk tree or kapok tree.[11]

Its different parts have been used for medicinal and non-medicinal purposes. In Southeast Asian countries, its leaves have been used to treat fever, cough, hoarseness, and venereal diseases. The bark has been employed in treating fever, asthma, gonorrhoea, and diarrhoea, and as an aphrodisiac, while the root has been used as a diuretic and febrifuge.[11,12,13]

C. pentandra plant contains a number of secondary metabolites which are assumed to be responsible for its promising medicinal properties like antioxidative[14], hypoglycaemic[15], anti-bacterial[16] anti-convulsant and antitumor activity.[17] Also in drug formulation, it is useful as a suitable excipient.[18]

Various studies have been done to determine both acute and subacute toxicity assessments of this plant using its various extracts in animal models. The findings consistently showed a low toxicity profile, with LD_{50} as high as 5000mg/ kg in rats suggesting that *C. pentandra* is relatively safe for consumption.[19] However its toxicity studies have never been carried out using alternative models like *Drosophila melanogaster*.

Therefore, this study aims to study the toxicity of C. pentandra acetone extract using *Drosophila melanogaster* model.

MATERIALS AND METHODS

Materials

Plant material: C. pentandra leaves were collected from Omu-aran in Kwara state. It was authenticated at the University of Ibadan Herbarium and given voucher number **UIH-22406.**

Chemicals and reagents: The chemicals, solvents and reagents used were of analytical grade (Sigma Aldrick, UK).

METHODS

Extraction of C. pentandra leaves

The leaves were air-dried, and extracted using the cold marceration method. The plant material was treated with petroleum ether to remove pigments and fats; soaked in water for 5 hours, boiled for 30 minutes and allowed to stand for 30 minutes so that the mucilage can be leaked into the water. The material was squeezed from the 8 folds of muslin cloth to remove the marc. Equal volume of acetone was added to the filtrate to remove the mucilage. The mucilage was separated, dried in the oven at 45ºC, sieved twice and stored in a dessicator.

Qualitative analysis of phytochemical constituents

Phytochemical screening of the different extracts were carried out using the standard procedures as described by[20]

Culture of *Drosophila melanogaster: Drosophila melanogaster* (Harwich strain), primarily sourced from the National Species Stock Center (Bowling Green, OH, USA) were cultured and maintained in the Drosophila Laboratory, Biochemistry Department, University of Ibadan, Nigeria as described by.[21]

Survival study: To determine the concentration and duration of exposure to *C. pentandra* leaf extract. The flies (both genders, 1-3 days old) were exposed to different concentrations (1 mg, 1.5 mg, 2 mg, 2.5 mg) respectively per 5 g diet over 14 days with daily mortality rate recorded and percentage survival determined.

The test was repeated using 37.5m,g, 75 mg and 150 mg per 5 g diet respectively.

Five groups of *Drosophila melanogaster* were used in subacute toxicity study of acetone extract of the leaves of *C. pentandra* and each group consists of 30 flies. The groups and treatment were designed as follows:

Group 1 - Control treated with water (0. 2 µl)
Group 2 - *C. pendantra* (1 mg/ 5 g diet, p.o.)
Group 3 - *C. pendantra* (1.5 mg/ 5 g diet, p.o.)
Group 4 - *C. pendantra* (2 mg/ 5 g diet, p.o.)
Group 5 - *C. pendantra* (2.5 mg/ 5 g diet, p.o.)

Group 1 - Control treated with water (0. 2 µl)
Group 2 - *C. pendantra* (37.5 mg/ 5 g diet, p.o.)
Group 3 - *C. pendantra* (75 mg/ 5 g diet, p.o.)
Group 4 - *C. pendantra* (150 mg/ 5 g diet, p.o.)

Statistical analysis: Statistical difference was determined by one-way analysis of variance (ANOVA) and $p<0.05$ was adjudged significant.

RESULTS AND DISCUSSION

Results

Qualitative analysis of C. pentandra leaf acetone extract.

Phytochemical Constituents	Results
Saponins	++
Tannins	+
Flavonoids	+
Cardiac glycosides	-
Steroids	++
Terpenoids	+
Phenols	+
Anthraquinones	+
Alkaloids	+

Survival Results

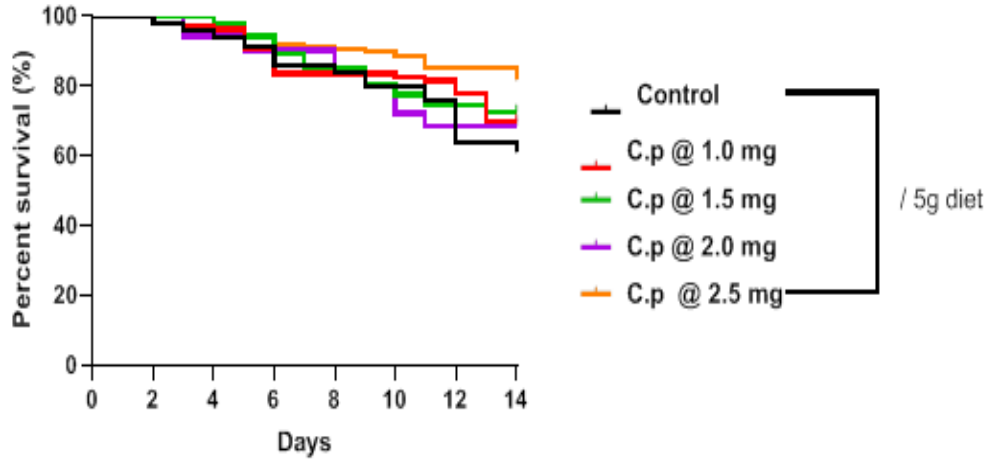

Fig. I: Survival of *Ceiba petandra* leaf acetone extracts at 1, 1.5, 2 and 2.5 mg per 5 g diet.

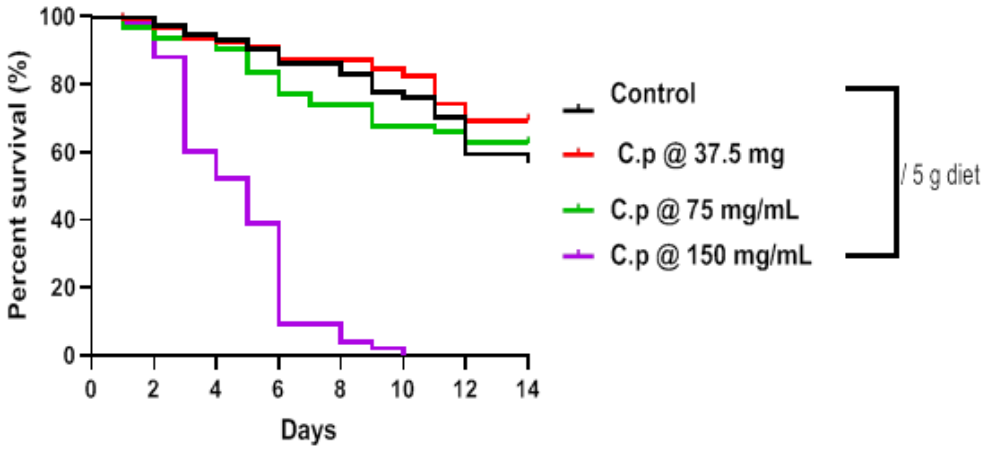

Fig. II: Survival of *Ceiba pentandra* leaf acetone extracts at 37.50 mg, 75 mg and 150 mg per 5 g diet.

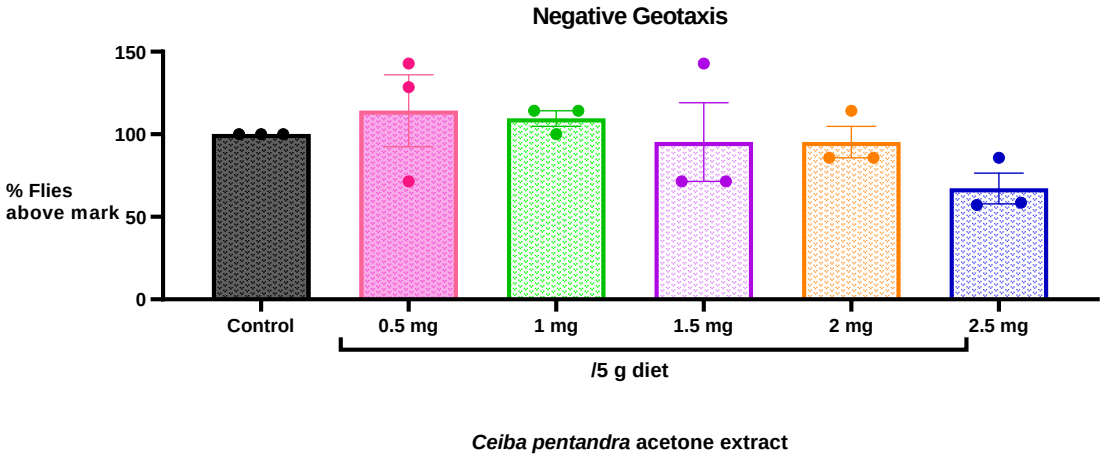

Fig. III: Bar graph comparing the negative geotaxis of the control to *C. pentandra* extract administered at different doses.

DISCUSSION

Phytochemical analysis

C. pentandra leaves contain various phytochemicals such as flavonoids, saponins, steroids, tannins, triterpenoids, alkaloids, glycosides, phenols, and resins, which have been confirmed through screening and metabolic profiling.[22,23]

The antioxidant property of this plant can be linked to the presence of flavonoids, phenols, and tannins which scavenge free radicals and reduce oxidative stress which inhibit inflammatory pathways.[24] To a certain extent, C. pentandra leaf acetone extract exhibits antimicrobial activity similar to literature due to the presence of alkaloids, saponins, and tannins because they have the ability to inhibit the growth of certain bacteria and fungi.[25] These phytochemicals are responsible for C. pentandra potential usefulness in managing oxidative-related diseases, microbial infections and inflammatory conditions.[26]

Survival of C. pentandra leaf acetone extracts

Figures 1 and 2 show the effect of varying concentrations of the acetone leaf extract of C. pentandra on the survival of D. melanogaster after a 14- day exposure. It was observed that at lower concentrations of <75mg/ 5 g diet, the survival rate was improved compared to that of the control. This survival rate increased with increase in concentrations (from 1mg/ 5 g diet to 70mg/ 5 g diet) of the extract, while showing a declining survival rate at values above 70 mg/ 5 g diet.

Negative geotaxis in Drosophila melanogaster, the innate behaviour of climbing against gravity when startled, has significant implications for understanding various biological and pathological processes. This behaviour is a valuable tool for studying locomotor function, aging and neurodegeneration.

Negative geotaxis declines with age in Drosophila, mirroring age-related locomotor impairments in humans. This decline is primarily due to reduced climbing speed, making it a useful model for studying aging and neurodegenerative diseases.[27]

It can be observed from the result obtained that C. pentandra acetone extract did not negatively impair the climbing activity of the flies at reduced doses but at a higher dose there was a slight reduction in this property showing that it is not likely to induce aging and neurodegenerative diseases at lower doses.

CONCLUSION

This study revealed that C. pentandra acetone extract exhibits a dose-dependent toxicity. Despite this plant has potential for pharmacological, pharmaceutical and nutraceutical applications, these findings highlight the importance of dose optimization. Furthermore, the suitability of D. melanogaster in assessing the toxicity of plant extracts was established.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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