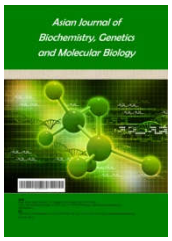

Asian Journal of Biochemistry, Genetics and Molecular Biology

5(2): 1-9, 2020; Article no.AJBGMB.60560
ISSN: 2582-3698

Determination of LD_{50}, Fecundity and Locomotor Effects of Methanol Root Extract of *Ximenia americana* Linn, in *Drosophila melanogaster*

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Authors' contributions

This work was carried out in collaboration among all authors. Author GDB conceived and designed the work. Author SO supervised the bench work and read-proved the manuscript. Authors GDB and WMI analyzed and typeset the work. Author SSG and MOU read proved and authorized the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2020/v5i230123
Editor(s):
(1) Dr. Arulselvan Palanisamy, Muthayammal College of Arts and Science, India.
Reviewers:
(1) Anna Carla Alberto-Silva, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Brazil.
(2) Tabe Franklin Nyenty, University of Yaounde 1, Cameroon.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60560

Original Research Article

Received 20 June 2020
Accepted 27 August 2020
Published 05 September 2020

ABSTRACT

Aim: The study aimed at assessing the LD_{50}, fecundity and locomotor effects of *Ximenia americana* L in *Drosophila melanogaster.*
Study Design: The study was an experimental design.
Place and Duration of Study: The study was done in the drosophila laboratory, Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria between November 2019 and Match, 2020.
Methods: The experimental animals (1-3 days old) of both sexes were exposed to different concentrations (1 mg 10 mg 50 mg, 100 mg, 200 mg, 250 mg 300 mg, 350 mg, 400 mg, 450 mg) of the plant extract per oral for seven days to determine the lethal dose, LD_{50}. Thereafter, five days

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treatment was done using 50 mg, 100 mg 200 mg and 300 mg concentrations of the extract to assay for fecundity and locomotor effect in the fruit fly.
Results: The LD_{50} of the methanol extract of *Ximenia americana* in *D. melanogaster* was found to be 327.7 mg, this showed that the plant extract is relatively safe. Also, the result showed that both fecundity and locomotor behaviour of the treated and untreated flies was not significantly ($P > 0.05$) different. Thus, the extract at the used concentrations does not affects significantly both the reproductive capacity and the motor functions in the fruit fly.
Conclusion: All the tested concentrations used in this research are relatively safe (because of high LD_{50} 327.7 mg) in the fruit fly and slightly increase the emergence of new fly with no noticeable negative effect in locomotor activity.

Keywords: Fecundity; Drosophila melanogaster; Ximenia americana; negative geotaxis.

1. INTRODUCTION

All living things, including plants and animals, naturally must reproduce i.e., give birth to young ones for the species to remain in existence. For most species (*Drosophila melanogaster* inclusive) two sexes (male and female) have to come together or mate [1] to achieve this task. Besides procreation, the coming together has both psychological, physical and social benefits [2] to the union.

Drosophila melanogaster (Fruit fly) is a holometabolous insect that has three life cycles: Embryo, larva, and pupa [3]. Drosophila belongs to the order Diptera, class Insecta, and like other members of the animal kingdom, for reproduction to occur there is usually a period of courtship, a behaviour observed in the fruit fly that involve the exchange of stimuli like visual, auditory and chemosensory signals between the male and female that could eventually lead to series of complex and well-coordinated motor behaviours culminating in successful copulation [4].

Fecundity is a measure of reproduction in Drosophila, which is an estimate of the number of the potentially viable embryo (eggs) laid by the animal, this can be a direct estimate of the number of young flies that emerged within a giving period, it is equally a widely used proxy for fitness estimation in animals [5,6].

Drosophila has been an acceptable model use in evolutionary biology because of some advantages it has over other animal models such as its functional homolog with humans [7], the ease-of-use, little space occupation and its short generation time i.e. its life cycle is short (11-12 days) depending on environmental factors. One very central advantage that is of interest to a biologist is, its characterization of life-history traits (e.g., life span, fecundity, mating competitiveness) [5].

In Drosophila, Fecundity is a highly plastic trait, strongly affected by factors like population density and the environment (temperature, relative humidity, nutrients, light), [8,9,10]. For a reliable comparison of fecundity data, it is essential to minimize the influence of these environmental factors by ensuring the maintenance of the experimental set-up under the same environmental factors for the generation(s) under study.

In order for us to measure the effectiveness of the plant extract on fecundity in Drosophila, we directly counted the number of young flies that emerged every 24 hrs after mating an equal number of exposed and unexposed male and female flies (1:1) and allowed them to lay eggs for 24 hrs.

For *Drosophila melanogaster* to meet its daily needs and carry out other natural activities that are inherent to the animal, it has to move around. Movement (locomotor behaviour) under the influence of tested substances in fruit fly can result from multiple causes, including genetic makeup, evolutionary constraints, and environmental impact [11]. Studies into the locomotor behaviour in the fruit fly can provide insights into understanding the animal internal physiological states as well as adaptive responses to external influence [12].

As in human beings, movement in model organisms, such as flight and locomotion in the fruit fly, is controlled by the central nervous system (brain), peripheral nervous system, and muscles [13,14].

To assay the effect of the plant extract on the movement or locomotor behaviour in the fruit fly, we employed the negative geotaxis(movement away from gravity) or climbing assay [15,16,17,18]. The general principle is to place a known number of treated and untreated (control)

flies of both sexes in a vial and tap the vial 3 times strongly against a hard surface, causing the flies to fall to the bottom of the vial. As it is an innate behavior to the fruit fly, the flies will attempt to climb to the top of the vial, oppose to gravity. This assay is quantitative and measures how many flies have climbed past a marker on the vial during an allotted time [19,15].

The plant *Ximenia americana* Linn. family Olacaceae, belong to the Kingdom, Plantae. Order, Satales. Genus, Ximenia. Species, *X. americana*. Synonym to this plant includes; Tallowwood, Yellow Plum and Sea Lemon in English. Other local names for this plant are Tsada (Hausa), Anya Nwona (Igbo), Igo (Yoruba), Chabbuli (Fulani) and Anomadze (Tiv). Globally, *Ximenia americana* L. is one of the eight species of Olacaceae family [20] that grows widely throughout the tropics in Africa, India and South East Asia to Australia, New Zealand, Pacific Islands, West Indies, Central and South America [21,19,20]. It is a plant of diverse habitats from semi-arid bushland, many types of dry woodland, sandy open woodland, coastal bushlands, and dry hilly areas and grows within altitude 900-2000 m [22,21]. It can also be found in coastal dunes, along with watercourses and on stony slopes where rainfall exceeds 500 mm per year. The plant grows in many soil types; but often on poor and dry soil conditions [23].

1.1 Ethno-Medical Uses

There is a wide report on the use of different parts of this plant in folk medicine for the treatment and management of many illnesses in humans and livestock [24]. Crushed bark is used for the treatment of hepatitis and malaria. Boiled and filtered pieces of bark in a tea glass are served for treatment of malaria, ulcer, leprosy skin infections and *Trypanosome congolense* [25,26,20]. Dried crush bark powder is applied on wounds surfaces for the treatment of infected wounds. Also, dried or fresh stem bark boiled in water is taking orally for the treatment of snakebite. Similarly, the bark is chewed to treat swelling pancreas and has been demonstrated to possess antioxidants activity [27,28,29].

The fresh leaves are used to treat bloody urine in livestock [21]. Further work on the plant has shown that it has anti-inflammatory activities and is believed to possess antineoplastic effect [30] and antimicrobial activities [21,31].

Also, Jose' et al. [9] reported on a wide range of biological activities of *X. americana*, to include, antimicrobial, antifungal, antitrypanosomal, antirheumatic, analgesic, molluscicide, pesticidal and also, have hepatic and hematological effects. Similarly, Agyigra et al. [20] reported on the gastroprotective effect of the stem bark of the plant. The root bark of this plant has shown to possess antitrypanosomal activity [32]. The roots are used by the Kagoro people (Aegworok) in Southern part of Kaduna State Nigeria for the treatment and management of sickle cell diseases [com].

The plant has fruits that are yellow-red edible drupe and oval in shape, approximately 2.5 cm in diameter. Each fruit contains one large endospermic seed within its green pulp. The seeds contain a small embryo near the tip, and a thin testa. They have up to 60% oil content and the seed coat ratio (seed coat mass/whole seed mass) is averagely 0.36 [23]. Elsewhere in Ethiopia, oil extracted from these plant seeds is used for lubrication, soap manufacture [22] and softening of leather [21]. Also, Feyssa et al. [21] and Abbink [33,34] reported on the use of this oil by Suri women in the country (Ethiopia) for contraception.

The use of different parts of this plant in managing different diseases has been demonstrated using rats/mice model. However, there is yet reported work that used the *Drosophila melanogaster* model to investigate the effects of *Ximenia americana* root extract on fecundity and locomotor capacity.

2. MATERIALS AND METHODS

2.1 Materials

The roots of the plant were collected in Makabun Village, Kagoro in Kaura Local Government Area, Kaduna State, Nigeria. The plant was identified by a plant taxonomist Mr. J. J. Azila of Federal College of Forestry Jos, Plateau State Nigeria, voucher number FHJ 243 was deposited at the herbarium of the college.

The plant roots were cleaned, the outer scale removed and air-dried at room temperature. The dried material was powdered using wooden mortar and pestle. The powdered sample was stored at room temperature in an airtight container and labeled for further work.

2.2 Drosophila Stock

The animal stock (Harwich strain of both sexes) used in this work was obtained from the Drosophila Laboratory Africa Center of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria. The flies were maintained and rare using corn meal food. The food contained, 100 gm of yellow-corn powder, 10 gm dry yeast (inactive and not hydrolyzed), 10 gm of agar-agar powder, 1 gm of methyl-4-hydrogen benzoate dissolve in 10 ml absolute ethanol and portable water adds to 1000 ml. All experiments were carried out using the same batch of corn powder, agar-agar powder, and Yeast. The fly's culture was maintained at 23 + 1°C, relative humidity ≈60% and 12 hours dark and light cycle.

2.3 Equipment and Reagents

The equipment used in the work include; Eppendorf centrifuge 5427 R, Jenway 7315 UV-Spectrophotometer, Metlar analytical balance MT-200B, glass stirring rod, beakers, plastic vails (50 cm height, 2 cm diameter), Rotary evaporator (RE-52A by PEC MEDICAL USA), hand magnifying lens, counting brushes, filter paper, cotton wool.

Reagents used include; Analytical grade methanol (CAS: 67-56-1, Lot: 1214788) by Fisher scientific UK, 70% ethanol, phosphate buffer saline (PBS), distilled water. All other solutions, chemicals and buffers used were prepared fresh using glass wires.

2.4 Methods

2.4.1 Dried plant material extraction (using 70% V/V methanol)

400 gm of pulverized *Ximenia americana* root was macerated in 4 L (1:10) of 70% v/v Methanol by cool extraction for 72 hours in an Amber bottle with occasional shaking. After the extraction period, the mixture in the bottle was filtered using Whatman No 1 filter paper, the filtrate was concentrated in a rotary evaporator at 48°C and later dried using a freeze dryer. The dried extract was finely powdered using porcelain mortar and pestle and stored in 50 ml sample bottles at 4°C in a refrigerator. The powder methanol extract was used subsequently to determine the lethal dose (LD_{50}), fecundity and locomotor effect using *the D. melanogaster* model.

2.4.2 Experimental design

Determination of LD_{50} and assessment of the effect of methanol root extract of *Ximenia americana* on the reproductive capacity (fecundity) and climbing behaviour of Drosophila was done using a short-term dietary regimen. Newly eclosed flies (both sexes), 1-3 days old were randomly divided into 5 separate groups, each group houses 60 flies in a 50 ml plastic vails and each group has 5 replicates were exposed to graded concentrations (50 mg, 100 mg, 200 mg and 300 mg) of the plant extract or without extract (control) for 5 days. The concentrations of the extract and duration for treatment employed in this work were predetermined from a pilot study (data not shown) and LD_{50} plot (Fig. 1). Newly emerged flies were used in this research work because it represents an important stage of organogenesis in animals. Thus, the developing organism is more fragile and susceptible to toxicants due to low and unmatured immunity and underdeveloped organ system compare to the adults, as such can easily be affected by any chemical change within its immediate surrounding environment [7].

- *Group 1*- Flies fed with food + 0 mg extract(control)
- *Group 11*- Flies fed with food + 50 mg extract(exposed)
- *Group 111*- Flies fed with food + 100 mg extract(exposed)
- *Group IV* – Flies fed with food + 200 mg extract(exposed)
- *Group V* – Flies fed with food +300 mg extract(exposed).

2.4.3 Determination of 7 days LD_{50}

The lethal dose (LD_{50}) was determined using a protocol previously described by Iorjiim et al. [35]. Briefly, sixty (60) flies (both sexes) of 1-3 day old were anesthetized under light ice, counted and exposed to 10 graded concentrations (1 mg, 10 mg, 50 mg, 100 mg, 200 mg, 250 mg 300 mg, 350 mg, 400 mg, 450 mg) of the plant extract and 1 ml distilled water (control) in 10 gm food for 7 days. Cumulative fly death was recorded every 24 hours for the duration of the treatment. The survival rate was subjected to dose-response simulation using GraphPad prism version 8. 0. 2 (623) for LD_{50} determination.

2.4.4 Five-day treatment for fecundity and negative geotaxis

From a 14 days survival curve (result not shown), 5 days treatment was set-up with flies' survival above 70% to determine short time effects of the plant extract on the reproductive (fecundity) and locomotor capacities in the fruit fly. The protocol previously described by Abolaji et al. [7] was adopted. Briefly, sixty flies aged 1-3 days old were treated with 50 mg, 100 mg, 200 mg, and 300 mg of the extract per 10 gm fly food respectively.

2.4.5 Reproductive effect

The fecundity of drosophila was studied after exposure of the flies to the test material according to the method previously described by Charpentier et al. [36] with little modification. Briefly, 10 flies (5- males, 5- females) were sorted out from each 5 days treatment group and control under ice anesthesia, the flies were allowed to recover fully for 20 mins and transferred into vials containing fresh fly food that has no treatment and allowed to mate and lay egg for 24 hours. After the allotted time (24 hours), the flies were removed. The experimental set up was observed every 24 hours for 14 days for the possible emergence of new flies. The average number of flies that emerged during the duration of the experiment (14 days) is a direct measure of the viable egg laid and hence, a measure of the effect of the plant extract in the fecundity of *Drosophila melanogaster* [6].

2.4.6 Negative geotaxis (Climbing) assay

The locomotor or climbing performance of *D. melanogaster* exposed to concentrations of *Ximenia americana* methanol root extract and control were investigated via negative geotaxis assay according to the protocol previously described by Abolaji et al. [7]. Briefly, 10 flies treated with the plant extract and untreated(control) were immobilized under light ice anesthesia and placed separately in vertical labeled glass vails (length, 15 cm; diameter, 1.5 cm) and allowed for 20 min to recover. Thereafter, the flies were gently tapped to the bottom of the column. In 6 s, the number of flies that climbed up to the 6 cm mark of the column, as well as those that remained below this mark was scored. Data obtained were expressed as the percentage of flies that escaped beyond the 6 cm mark in 6 s. The score of each group was an average of three independent trials for each group of treated and control flies.

2.5 Statistical Analysis

The data were expressed as mean±SEM. The analysis was carried out using (ANOVA) followed by Tukey's posthoc test to identify statistically different test groups. GraphPad Prism statistical software version 8.0.3(623) was used. The results were considered statistically significant at $P< 0.05$.

3. RESULTS AND DICUSSION

3.1 7 Days LD_{50}

The lethal dose (LD_{50}), defined as the concentration of test material in a normal fly food (media) that cause 50% of fly death in 7 days [37]. The concentration for use in the research was obtained from exposing the flies to ten staggered concentrations (stated above) of the plant extract for 7 days. Thereafter, the LD_{50} of the plant extract was determined (Fig. 1) to be 327.7 mg. This value of LD_{50} shows that the extract is relatively safe.

3.2 Fecundity

The fecundity of the treated and untreated flies (Fig. 2) did not show any difference. This means that while taking this plant extract, patients' reproductive capacity is not affected negatively since both the exposed and unexposed groups were able to emerge appropriately.

3.3 Locomotor Effect

The ability of the treated and untreated flies to climb against gravity (Fig. 3) was not altered by the administration of the plant extract. This further demonstrated the safety of this plant material on motor co-ordination in D*rosophila melanogaster.*

3.4 Discussion

The concentration of methanol root extract of *Ximenia americana* that is capable of killing 50% (LD_{50}) of the population in *D. melanogaster* was found to be 327.7 mg/10 g fly medium. This value showed that the extract is relatively safe. This is a confirmation of the used of this plant material for different purposes in folk medicine [21,38].

From the result, we discovered that the plant extract did not alter the reproductive capacity of the experimental animal. Hence, there was no

significant difference (*P*> 0.05 vs control) between the treated groups as compared to the control. Though the result showed that at 200 mg concentration, demonstrated a slide increased in the percentage of flies that eclosed when compared to the control (and other concentrations). But the difference was not significant (*P*> 0.05). This observed increase in fertility in *D. melanogaster* by the plant extract may in part suggests why oil from this plant is used to improved fertility by local women in Ethiopia [21,33,34].

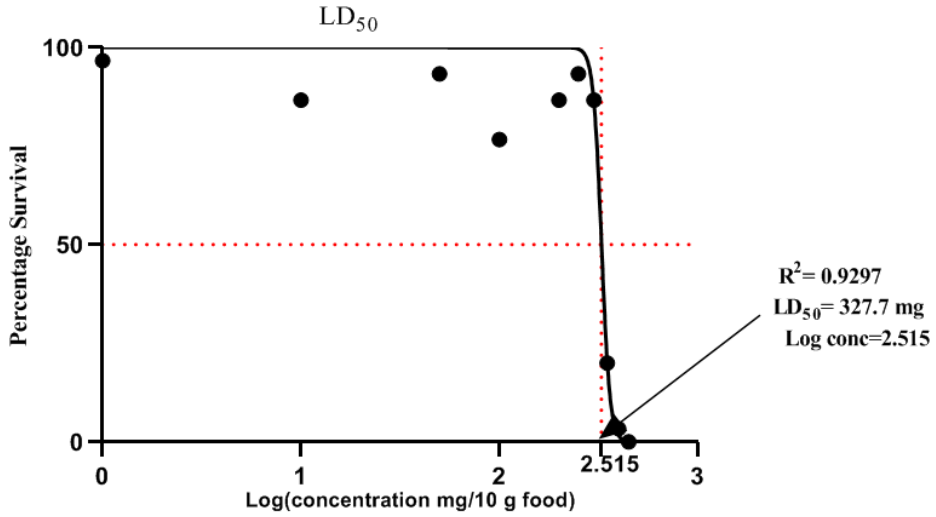

Fig. 1. LD_{50} of methanol extract of *Ximenia americana* in *D. melanogaster*
Data are presented as mean± of five independent biological replicates

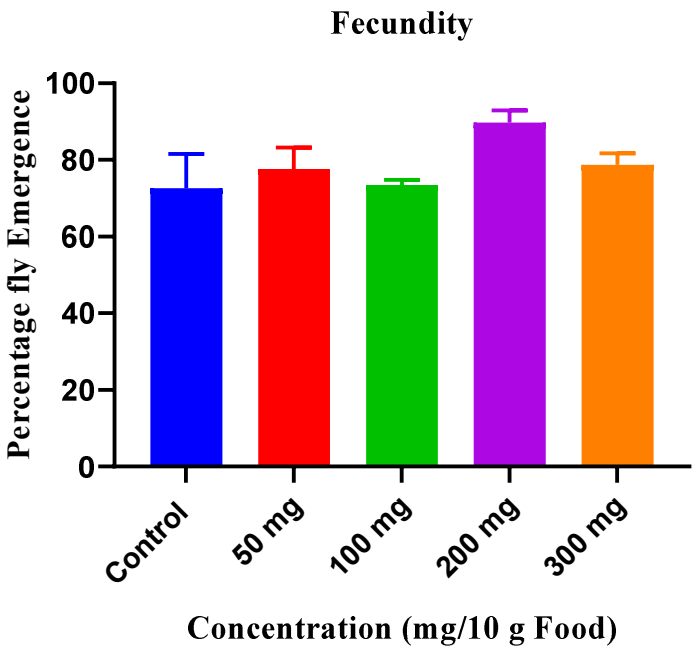

Fig. 2. Effect of methanol extract of *Ximenia americana* on reproductive capacity in *D. melanogaster*
The methanol extract of Ximenia americana did not (P< 0.05 VS Control) significantly impair Fecundity in D. melanogaster
Data are presented as ±SEM of five independent biological replicates. Each assay was carried out in two independent experiments

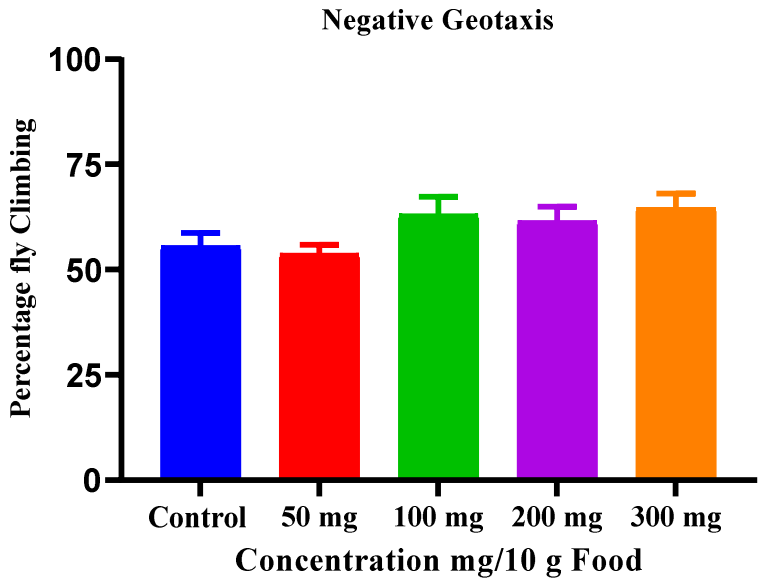

Fig. 3. *Ximenia americana* methanol extract did not significantly (P > 0.05 vs control) impaired the climbing behavior in *Drosophila melanogaster* when compared to the control

Data are presented as ±SEM of five independent biological replicates. Each assay was carried out in two independent experiments

Similarly, the extract increased the locomotor activity in *D. melanogaster* when compared with the control. However, the difference was not statistically significant (*P*> 0.005) when compared to the control. This also, demonstrated that the plant material does not affect motor coordination in the experimental animals.

4. CONCLUSION

This work revealed and validate the safety of *X. americana*. The work further explains why this plant is very popular in the treatment of different disease conditions particularly among the Hausa/Fulani and the Aegworok tribes in the northern part of Nigeria [39,40,26,41].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/60560