



Toxicity Assay of Methanolic Extract of *Caryota No* Seed using *Drosophila melanogaster* Model

Chinonye A. Maduagwuna^{1,2*}, Simeon Omale^{1,3}, Monday A. Etuh³ and Steven S. Gyang¹

¹Department of Pharmacology and Toxicology, University of Jos, Jos, Nigeria.

²Department of Pharmacology and Therapy, University of Abuja, Abuja, Nigeria.

³African Centre of Excellence for Phytomedicine Research and Development, University of Jos, Jos, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author CAM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SO and MAE managed the analyses of the study. Author SSG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: To investigate the effect of methanolic extract of *Caryota no* (CN) seeds in *Drosophila melanogaster* (DM) survival and life span.

Study Design: Experimental design.

Place and Duration: African Centre of Excellence for Phytomedicine Research and Development, University of Jos, Jos Plateau State Nigeria between June 2018 and February 2019.

Methods: The LC₅₀ was determined by exposing 50 flies to concentrations ranging from 1 mg to 600 mg per 10 g diet and mortality of flies was scored every 24 hours for 14 days and from the results, five doses were chosen for the next assay. Survival assays were carried out by exposing 50 flies in each vial to the following concentrations: 300 mg, 350 mg, 400 mg, 500 mg and 600 mg of methanolic extract in 5 replicates for 28 days with daily recording of mortality

*Corresponding author: E-mail: elchinonye@gmail.com;

while the longevity assay continued from the survival until the last fly dies. All three experiments were done as three independent trials.

Results: The LC₅₀ values of the methanolic extract was determined to be 6.533e+017 mg/10g food in *D. melanogaster*. The result of the survival assay with methanolic extract of CN showed slight significant ($P < .05$) increase with the lowest two doses but no significant ($P > .05$) difference with other higher doses compared to the control. The longevity assay revealed that the extract significantly ($P < .05$) decreased longevity in *Drosophila melanogaster*.

Conclusion: The results obtained from evaluating the methanolic extract of *Caryota no* indicate that the plant is relatively non-toxic and maybe safe under acute and subacute exposures but may become deleterious during chronic exposure.

Keywords: Survival; longevity; *Caryota no*; toxicity; *Drosophila melanogaster*; LC₅₀.

1. INTRODUCTION

Medicinal herbs have consistently been considered the leading source of pharmaceuticals, employed in the treatment of various human diseases due to their high chemical diversity and broad biological functionality [1]. Traditional medicines are mostly compounded from natural products therefore there is a likelihood of them being accepted by the body better than synthetic substances [2] and have been recognized to have convincing and credible curative effects [3].

Some researchers [4] illustrated by their work that mice infected with *P. aeruginosa* and treated with a garlic/tobramycin combination showed significantly improved clearing of their bacterial infections as compared to a placebo control group. In vitro analysis of *P. aeruginosa* biofilms showed considerable destruction of the biofilm when exposed to a combination of garlic extract and tobramycin. Exposure to either compound alone had little or no effect on the biofilm. Another group [5] also demonstrated that S-phenyl-L-cysteine sulfoxide and its breakdown product, diphenyl disulfide, significantly reduced the amount of biofilm formation by *P. aeruginosa*. It was also found that a tannin-rich component of *Terminalia catappa* leaves (TCF12) was able to inhibit the maturation of biofilms of *P. aeruginosa* to significant levels [6]. A large number of plant products have been proven to be very valuable for the treatment of a myriad of medical maladies. Natural toxicants present in human foods and animal feeds present a potential hazard to health. The starting point in determination of the safety profile of any compound is in the determination of its lethal concentration (in acute or chronic conditions) and also to check other toxicological parameters using experimental animals or insects. Lethal Concentration 50 or LC₅₀ is a standard measure

of toxicity to determine how much of a substance is needed to kill half of a group of experimental organisms in a given time [7]. These preclinical studies must be undertaken before any biologically active substance must be evaluated clinically for therapy.

The common fruit fly, *Drosophila melanogaster*, has been extensively studied for decades. In effect, it was introduced as a decisive model in biology about a century ago. The fly shares several basic biological, biochemical, neurological and physiological similarities with mammals. It is documented that about 75 % of human disease-causing genes have functional homolog in DM [8]. The fly can effectively be maintained at low cost in the laboratory, and it has been recommended as an alternative model to vertebrate usage. Consequently, it has attracted the attention of toxicologists [8]. Determination of LC₅₀ of any substance in *D. melanogaster* is essential for selection of the concentration of the substance for further experiments.

Caryota no palm is reported to be one of the largest species of the genus found in Borneo rainforests. The common name is the Giant Fishtail Palm [9]. In habitat, this palm can reach a height of 75 inches and stems measure 18-20 inches in diameter [9]. *Caryota* species, mostly found in Asia, are used traditionally in the treatment of gastric ulcer, migraine headaches, and snakebite poisoning and also rheumatic swellings by preparing porridge from the flowers [10]. What sets this palm apart from others in the genus is its upright growth habit. Although this palm is considered a giant, its footprint in the landscape is reduced by its fronds growing mostly upward and rarely ever extending horizontally from the stem. This palm would grow successfully anywhere a coconut palm thrives. CN is not wind resistant. Along with *Arenga*

pinnata, it is one of the least wind resistant palms. These researchers recorded that *Caryota urens* (which is from the same family of palms) is suggested to treat seminal weakness and urinary disorders [11]. Very scanty information has been reported concerning research works on CN.

The aim of this work is to do a preliminary screen of methanolic extract of *C. no* for LC₅₀, and their effects on survival and longevity of *D. melanogaster* so as to determine its toxicity profile and to establish a base line for future studies on the plant.

2. MATERIALS AND METHODS

2.1 Reagents

All chemicals used were of analytical grade. Methanol and Distilled water were obtained from Africa Centre of Excellence in Phytomedicine Research and Development, Jos, Plateau State, Nigeria.

2.2 Plant Collection and Preparation

The plant material was collected from Games Village, Abuja, Nigeria. The plant was identified by a taxonomist in the herbarium of the Federal college of Forestry Jos. The seeds were sorted, air-dried for several days and then pulverized to powder using a commercial grinding machine. The soxhlet extractor was used for extraction of the plant compound using analytical grade 80 % methanol as solvent following a method described by Virost et al., [12]. A rotary evaporator was employed to recover the solvent. The extract was further dried in a water bath regulated at 40°C, while the extract was exposed to a freeze drier and kept in an airtight container.

2.3 Fly Strains

D. melanogaster Harwich strain was obtained from Africa Center of Excellence in Phytomedicine Research and Development, University of Jos and maintained at constant temperature and humidity (23 °C; 60 % relative humidity, respectively) under 12 h dark/light cycle. The flies were cultured by feeding them with a standard medium of the following compositions; 1700 ml of water, 16 g agar agar, 20 g of baker's yeast, 100 g of corn flour, and 1 g of methyl paraben dissolved in 5 ml of absolute ethanol, 1700 ml of water [13].

2.4 LC₅₀ of Methanolic Seed Extract of CN

The 14- days LC₅₀ was determined following the method described [7] with slight modification. 50 flies (of both genders (1–3 days old) per vial were exposed to the following concentrations; 1 mg, 10 mg, 50 mg, 100 mg, 250 mg, 300 mg and 350 mg of methanolic extract of *Caryota no* seed per 10 g diet. Mortality of flies was scored every 24 hours for 14 days. During the experimental period, flies were transferred onto new vials containing fresh food every 2 days. Details are stated in 2.5 and 2.6.

2.5 Survival Assay of Methanolic Seed Extract of CN-treated Flies

50 flies of both genders (1–3 days old) were exposed to selected concentrations of methanolic extracts of CN seeds (300mg 350mg, 400mg, 500mg and 600mg prepared in distilled water) in five replicates for 28 days [14,15]. The numbers of live and dead flies were scored daily till the end of the experiment and the survival rate was expressed as percentage of live flies.

The flies were divided into six groups containing 50 flies each. Control group was placed on normal diet alone while groups II–IV were placed on basal diet containing methanolic seed extract of CN at various concentrations of diet as shown thus;

Control group	Basal diet
300 mg group	Basal diet + 300mg CN methanolic seed extract/10g fly food
350 mg group	Basal diet + 350mg CN methanolic seed extract/10g fly food
400 mg group	Basal diet + 400mg CN methanolic seed extract/10g fly food
500 mg group	Basal diet + 500mg CN methanolic seed extract/10g fly food
600 mg group	Basal diet + 600mg CN methanolic seed extract/10g fly food

During the experimental period, flies were transferred onto new vials containing fresh food every 2 days. The flies were exposed to these treatments for 28 days, and the vials containing flies were maintained at room temperature. All experiments were carried out in triplicate (each experimental group was carried out in five independent vials). Survival analyses were calculated based on the number of deaths recorded and evaluated by the log-rank Mantel-Cox test.

2.6 Longevity Assay of Methanolic Seed Extract of CN-treated Flies

Longevity assay proceeded as a continuum from the survival assay [16,17], such that after 28 days, the daily recording of number of deaths continued until the last fly dies. Survival analyses were calculated based on the number of deaths recorded and evaluated by the log-rank Mantel-Cox test.

2.6.1 Maintaining the experiment

The vials containing fresh food were made to be at room temperature for each transfer. During the experimental period, flies were transferred onto new vials containing fresh food every 2 days. This step will ensure that the feeding environment for young females is not disrupted by the presence of larvae. This transfer were completed without anesthesia, which can induce acute mortality, particularly in older flies (Pletcher, personal observations).

During each vial transfer, the dead flies in the old vial were counted, and the dead flies that are carried to the new vial also noted. This information was recorded separately in two columns in a spreadsheet. This will ensure that the carried flies are not double-counted. The total number of deaths (dead + carried) should at least equal the number of carried flies from the previous transfer. Subtract the number of previously carried flies from the total number of deaths to determine the number of new deaths.

A fly is considered right-censored if it left the experiment prior to natural death through escape or accidental death. Animals exiting the experiment in this way were entered into a separate column on the day that the fly exited the experiment. Censored flies are not recorded as dead.

These transfer steps were continuously repeated until the last survivor is dead. As the flies age, some flies may lie on their back and appear dead due to their inactiveness. Therefore when counting carried (dead) flies, the side of the vials were tapped to determine if there are leg movements. If so, these flies are still alive. In the case where flies remain stuck to the food in the old vial but alive, they should not be counted as dead but were rescued by further tapping of the vial to dislodge the fly. Censoring such flies should be used with caution as it may result in experimental bias.

2.7 Statistical Analysis

Analysis of the data was done for the determination of the LC_{50} of CN on adult *D. melanogaster*. The data was expressed as mean \pm SEM (standard error of the mean) of five parallel measurements, and the statistical analysis was carried out using one-way analysis of variance (ANOVA) and two-way ANOVA in cases of comparisons with the software, GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA). The results were considered statistically significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 LC_{50} of Crude Methanolic Seed Extract of CN

14 days LC_{50} of methanolic extracts of CN seeds revealed that the concentration that can kill 50 % of flies was found to be $6.533e+017$ mg/10 g diet (Fig. 1).

Exposure to methanolic extract of CN seeds resulted in LC_{50} levels of $6.533e+017$ mg/10g food in DM. The LD_{50} of the methanolic extract in swiss rats was also determined to be > 5000 mg/kg by the oral route and >1000 mg /kg by the intraperitoneal route (unpublished). The concentration that can kill 50 % of the test organism, LC_{50} agrees with the unpublished LD_{50} value obtained from animal studies. This high level of LC_{50} suggests the safety of this extract and also serves as a baseline for selecting the concentrations; 350 mg, 400 mg and 500 mg per 10 g diet for 28-days survival study. It can therefore be inferred that the methanolic extract of CN seeds is relatively safe.

3.2 Percentage Death of Flies Treated with Methanolic Seed Extract of CN

The survival result (Fig. 2) for the methanolic extract show statistically significant ($P = .007$ **) decrease in death by the treatment groups compared to the control. The lowest extract dose recorded the highest number of deaths. The higher doses lowered the percentage death. The difference observed was later found not to be between the control and the lowest two groups but rather in comparison between the two said groups. There was rather a significant difference (**) between the lowest extract dose (300 mg/10g food) and the immediate next dose (350

mg/10g food) – the difference ($P = .009$) observed was between the last two lowest treatment doses. It can therefore be inferred that

exposure to methanolic extract of CN caused significant ($P < .05$) effect on percentage deaths in DM.

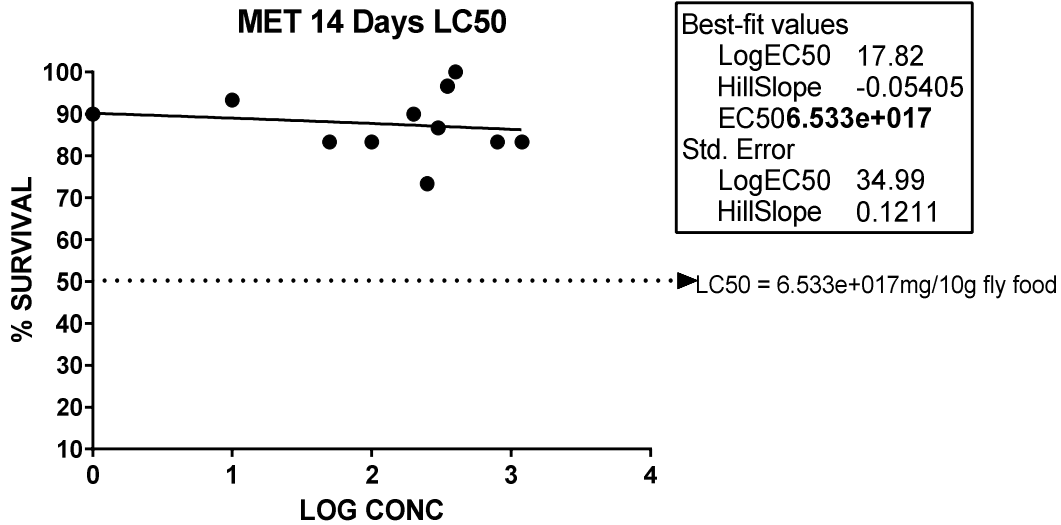


Fig. 1. LC₅₀ of methanolic extract of CN seeds on *D. melanogaster*

The scattered dots representing the expected percentage of fly death are joined to form a trend line which help determine the log concentration of the extracts corresponding to 50% fly death. The LC₅₀ of CN in log₁₀ scale is between 1 to 3.079. The Hillslope value for the LC₅₀ in 95% confidence interval is found to be in the range -0.489 – 0.176 mg/10g food. Data presented as mean ± SEM of five independent biological replicates of the extract concentration (n =50)

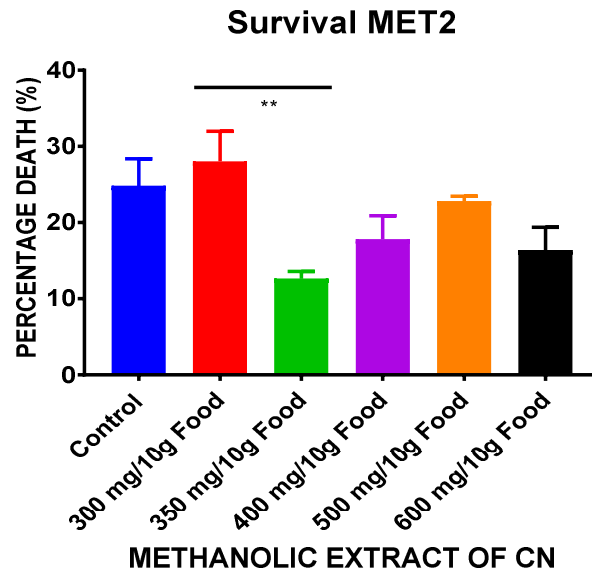


Fig. 2. Percentage deaths in *D. melanogaster* after treatment with methanolic extract of CN

Data presented as mean ±SEM of five independent biological replicates of the extract concentration (n =50). Extracts: significant from control * $P < 0.05$; ** $P < 0.001$; *** $P = 0.0002 - 0.0004$; **** $P < 0.0001$

3.3 Survival Assay of Methanolic Seed Extract of CN-treated Flies

Exposure to methanolic extract of CN caused nonsignificant ($P = .672$) effect on survival in DM (Fig. 3). By the 28th day, the survival proportions for the control, 600, 500, 400, 350, and 300 mg/10g diet groups were 23, 4.7, 1.7, 0, 10.0, 2.1 percent respectively. Also, the number of subjects at risk in the same order at the 28th day of the assay were 10, 10, 10, 10, 10, and 10. For the summary of the data, the median survival is 9, 18.5, 20, 19, 11, and 18 for the groups. It can be inferred that the methanolic extract of CN did not increase or decrease survival of DM adult fly after 28 days oral exposure.

3.4 Longevity Assay of Methanolic Seed Extract of CN-treated Flies

The graph (Fig. 4) illustrates that the methanolic extract caused a significantly ($P < .0001$) reduced life span in *D. melanogaster* between the groups and the control. The graph illustrates that there was statistically significant difference between the groups and the control.

By the 38th day, the survival proportions for the control, 600, 500, 400, 350, and 300 mg/10g diet groups were 34, 6.4, 0, 14.8, 0, 7.2 %

respectively. Also, the number of subjects at risk in the same order at the 38th day of the assay were 87, 46, 8, 38, 52, and 20. For the summary of the data, the median survival is 29, 32, 30, 31, 34, and 25 for the groups. The lowest extract dose also recorded the least number of median survival. The higher doses were better tolerated. The methanolic extract shortened the fly life span in comparison to the control ($P < 0.0001$). The last control fly died at day 54 while most of the treatment groups could not survive to 45th day. A particular group all died by day 39 and this is an obvious shortening of life span on chronic exposure in comparison to the control.

Exposure to methanolic extract of CN seeds significantly ($P < .05$) decreased life span in *D. melanogaster*. The comparison of longevity data between the nhexane extract of CN which was also researched on by this author and methanolic extract of CN is shown in Table 1.

The table shows a comparison between the two extracts in longevity in DM. The highest dose of the nhexane extract sustained fly life for 70 days while that of the methanolic extract could only sustain fly life for 40 days. For the lowest doses, it was 59 and 39 days for nhexane and methanolic extracts respectively. The middle doses were 68, 68 and 65 days for nhexane and 38, 40 and 38 days for methanolic extract.

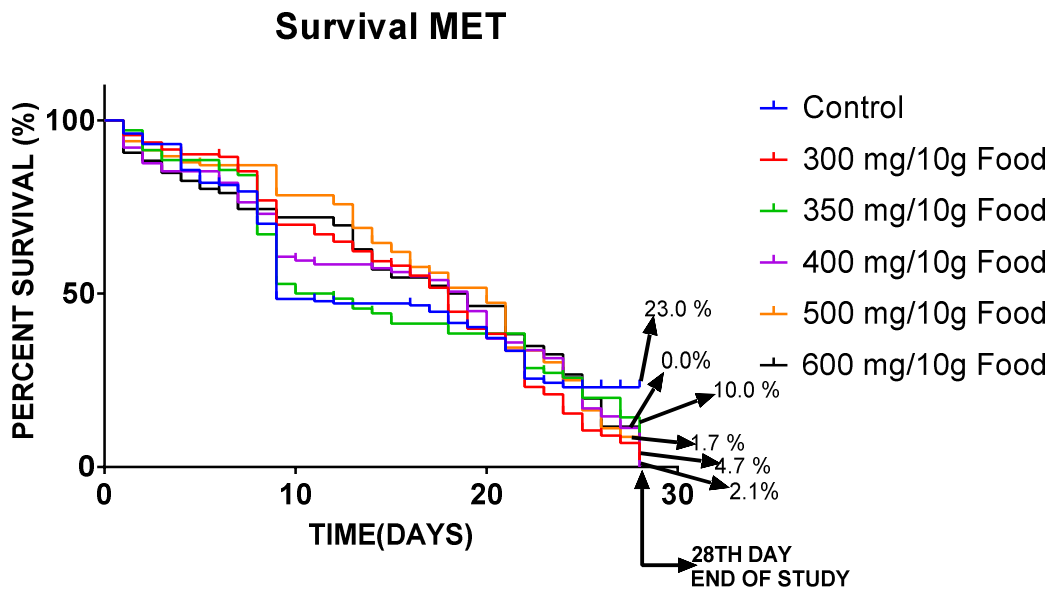


Fig. 3. Effect of methanolic extract of CN on survival of *D. melanogaster*
 Data presented as mean \pm SEM of five independent biological replicates of the extract concentration ($n = 50$).
 Extracts: significant from control

Longevity MET

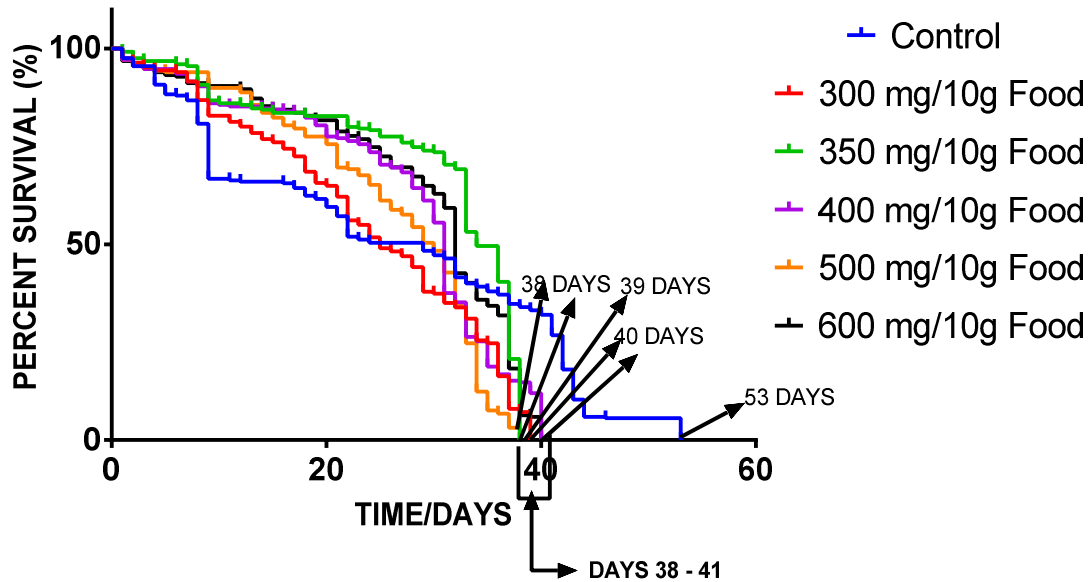


Fig. 4. Effect of methanolic extract of CN on lifespan of *D. melanogaster*

Data presented as mean \pm SEM of five independent biological replicates of the extract concentration (n=50).
 Extracts: significant from control * P < 0.05; ** P < 0.001; *** P = 0.0002 - 0.0004; **** P < 0.0001

Table 1. Effect of extracts of CN on longevity of *Drosophila melanogaster*

Drug Conc	nhexane Time(Days)	Methanol Time (Days)
Control	69	53
600mg/kg	70	40
500mg/kg	68	38
400mg/kg	68	40
350mg/kg	65	38
300mg/kg	59	39

3.5 Discussion

The toxic effect of any substance following a single acute exposure may be quite different from the effects produced by chronic exposure. It was reported [7] that a small amount of cryolite at one-time application is not sufficient to produce detectable changes in the biology of the animal, while the same small amount of the chemical applied day after day may cause chronic illness and ultimate death. At lower concentrations, insects try physiologically to combat the poisonous effects of any chemical by its elimination through the intestinal tract. Rapid elimination of cryolite from the rat digestive tract can save the animal from harmful effects [18]. A similar response is also true for

Drosophilids. It was reported that *drosophila* larvae which were exposed to 65,000 to 70,000 μ g/ml cryolite through food showed 50% mortality after 18 hours of acute exposure, whereas only 150 to 160 μ g/ml cryolite was sufficient to cause 50% mortality in case of chronic exposure [7]. The very high value of LC₅₀ recorded (Fig. 1) infers that the methanolic extract is very safe.

The results (Fig. 2) agrees with the review [14] which refers to [19] which have proven that the fruit fly tolerates only up to 1% of fat in the diet. It was observed [15] that higher dietary inclusions of *Garcinia kola* seed reduced the survival rate of *D. melanogaster* more significantly compared to control flies. This agrees with what was observed

by this researcher that different doses of dietary inclusions could prolong or reduce survival.

The phytochemical analysis of the methanolic extract of CN revealed a very high quantity of carbohydrates and some level of saponins (unpublished). This information is also supported by a research work [20] which concluded that diets rich in excess carbohydrates (saccharides) tend to lower the life span of flies and also [15] which showed that saponins are toxic to *Drosophila* and decreased the levels of acetylcholinesterase (AChE) activity.

Diet such as cornmeal prolongs the lifespan of the fly, while diets with high quantities of free available carbohydrates (saccharides) and cholesterol can reduce life expectancy [20]. In addition, overcrowding has been shown to reduce the longevity of the fly [21]. The similarities of molecular processes involved in the control of lifespan and aging between DM and humans, coupled with good degree of genetic homology between the two species, makes *D. melanogaster* an interesting model system for toxicologists. It can be inferred that on the overall that the methanolic extract of CN is safe.

On the whole, is there really a reduction in longevity since the average life span of fruit fly is between 40-120 days?

4. CONCLUSION

From the findings it can be concluded that methanolic extract of CN seeds has high $LC_{50} = 6.533e+017$ mg/10 g diet; caused a significant increase and decrease in survival with the 300mg/10g food and 350 mg/10g food respectively and no significant changes with higher doses of the extract; however it could only sustain fly life for only 40 days and could imply that it is safe in acute and subacute but maybe deleterious in chronic exposure.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Therasa SV, Thirumalai T, Tamilselvan N, David E. In-vivo and ex-vivo inhibition of intestinal glucose uptake: A scope for antihyperglycemia. *Journal of Acute Disease*. 2014;3(1):36–40. Available: [https://doi.org/10.1016/S2221-6189\(14\)60008-1](https://doi.org/10.1016/S2221-6189(14)60008-1)
2. Egu M, Etuk E, Bello S, Hassan S. Isolation and structural characterization of the most active antidiabetic fraction of *Corchorus olitorius* seed extract. *Journal of Advances in Medical and Pharmaceutical Sciences*. 2015;2(3):75-88. Available: <https://doi.org/10.9734/jamps/2015/14855>
3. Sofowora A, Ogunbodede E, Onayade A.. The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary, and Alternative Medicines: AJTCAM / African Networks on Ethnomedicines*. 2013;10:210–229. Available: <https://doi.org/10.4314/ajtcam.v10i5.2>
4. Ahmad I, Husain FM, Maheshwari M, Zahin M. Medicinal Plants and Phytocompounds: A Potential Source of Novel Antibiofilm Agents. 2014;205-232. Available: https://doi.org/10.1007/978-3-642-53833-9_10
5. Cady NC, McKean KA, Behnke J, Kubec R, Mosier AP, Kasper SH, Musah RA. Inhibition of biofilm formation, quorum sensing and infection in *Pseudomonas aeruginosa* by natural products-inspired organosulfur compounds. *PloS One*. 2012;7(6):e38492. Available: <https://doi.org/10.1371/journal.pone.0038492>
6. Taganna JC, Quanico JP, Perono RMG, Amor EC, Rivera WL. Tannin-rich fraction from *Terminalia catappa* inhibits quorum sensing (QS) in *Chromobacterium violaceum* and the QS-controlled biofilm maturation and LasA. Elsevier. *Journal of Ethnopharmacology*. 2011;134(3):865-871. Available: <https://www.sciencedirect.com/science/article/pii/S0378874111000420>
7. Podder S, Roy S. Exposure-dependent variation in cryolite induced lethality in the non-target insect, *Drosophila melanogaster*. *Interdiscip Toxicology*. 2014;7(1):17–22.
8. Abolaji AO, Kamdem JP, Farombi O, Batista J, Da Rocha T. *Drosophila*

- melanogaster* as a promising model organism in toxicological studies: a mini review neurotoxicity of methylmercury in insects view project science education view project. Arch Bas App Med. 2013;1:33-38.
Available: <https://www.researchgate.net/publication/259184998>
Accessed: 14 December 2019.
9. Koopman J, Simpson D, Goetghebeur P, Wilson K, Egorova T, Bruhl J, Govaerts R. World checklist of Cyperaceae. Facilitated by the Royal Botanical Gardens, Kew. 2005;1-223.
Available: <http://apps.kew.org/wcsp/>
Accessed 14 October 2014
 10. Vanaja D, Kavitha S. A Study on the Bio-efficacy of *Caryota urens* L. World Journal of Pharmaceutical Research. 2017;6(4): 1381-1398.
 11. Charles A, Joseph M, Ramani VA, Joseph S, Nadu T. Quantitative estimation of primary and secondary metabolites on flowers of *Caryota urens*. L. International Journal of Applied Biology and Pharmaceutical Technology. 2011;(3):431-435.
Retrieved: https://www.researchgate.net/publication/305073434_Qualitative_phytochemical_screening_antioxidant_and_antimicrobial_activity_studies_on_ethanolic_flowers_extract_of_caryota_urens_LINN
 12. Virost M, Tomao V, Ginies C, Chemat F. Total lipid extraction of food using d-limonene as an alternative to hexane. Chromatographia. 2008;68(3-4):311-313.
 13. Etuh MA, Aguiyi JC, Ochala SO, Simeon O, Oyeniran OI, Debola OO, Pam D. The In vivo antioxidant protective activity of *Mangifera indica* cold aqueous leaf extract in *Drosophila melanogaster*. Journal of Advances in Biology & Biotechnology. 2019;22(2):1-7.
 14. Abolaji AO, Kamdem JP, Lugokenski TH, Nascimento TK, Waczuk EP, Farombi EO, Loreto EL, Rocha JB. Involvement of oxidative stress in 4-vinylcyclohexene-induced toxicity in *Drosophila melanogaster*. Free Radic Biol Med. 2014; 71(6):99-108.
Available: <https://doi.org/10.1016/j.freeradbiomed.2014.03.014>
 15. Oboh G, Ogunsuyi OB, Ojelade MT, Akomolafe SF. Effect of dietary inclusions of bitter kola seed on geotactic behavior and oxidative stress markers in *Drosophila melanogaster*. Food Science and Nutrition. 2018;6(8):2177-2187.
Available: <https://doi.org/10.1002/fsn3.782>
 16. Abolaji AO, Olaiya CO, Oluwadahunsi OJ, Farombi EO. Dietary consumption of monosodium L-glutamate induces adaptive response and reduction in the life span of *Drosophila melanogaster*. Cell Biochemistry and Function. 2017;35(3): 164-170.
Available: <https://doi.org/10.1002/cbf.3259>
 17. Linford NJ, Bilgir C, Ro J, Pletcher SD. Measurement of lifespan in *Drosophila melanogaster*. J Vis Exp. 2013;(71):50068. DOI: 10.3791/50068
 18. Largent EJ. The comparative toxicity of Cryolite for rats and rabbits. J Industry Hyg. 1948;30:92-97.
 19. Miquel J, Fleming J, Economos AC. Antioxidants, metabolic rate and aging in *Drosophila*. Archives of Gerontology and Geriatrics. 1982;1(2):159-165.
Available: [https://doi.org/10.1016/0167-4943\(82\)90016-4](https://doi.org/10.1016/0167-4943(82)90016-4)
 20. Hirth F. *Drosophila melanogaster* in the Study of Human Neurodegeneration. CNS & Neurological Disorders - Drug Targets. 2010;9(4):504-523.
Available: <https://doi.org/10.2174/187152710791556104>
 21. Joshi A, Mueller LD. Adult crowding effects on longevity in *Drosophila melanogaster*: Increase in age-dependent mortality. Curr Sci. 1997;72(4):1225-260.

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