Trop J Nat Prod Res, July 2025; 9(7): 3271 - 3279 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Safety Assessment of the Crude Methanol Extract of *Sarcocephalus latifolius* (Sm.) E. A. Bruce Fruits in *Drosophila melanogaster*

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ARTICLE INFO

Article history:
Received 17 February 2025
Revised 23 May 2025
Accepted 28 May 2025
Published online 01 August 2025

ABSTRACT

Healing with medicinal plants is as old as mankind itself. The fruit of *Sarcocephalus latifolius* (SL) is used in folk medicine to treat tumours and other related diseases. This study aimed to assess the safety of the crude methanol extract of *Sarcocephalus latifolius* (Sm.) E.A. Bruce fruits (MSL) in *Drosophila melanogaster*. The study was performed in the *in vivo* mode. Adult Wild type (Harwich strain) flies were divided into six groups of 50 flies each. Group 1 (control, vehicle only), groups 2 - 6 (treated with 0.1, 0.2, 0.5, 1, and 10 mg/kg MSL diet, at the stated doses, respectively). The flies were exposed to the various treatments for 7 days, initially to assess survival status, and later to 28 days. Thereafter, the behavioural, inflammatory, oxidative stress, antioxidant status, eclosion rate and biochemical parameters were assessed using standard methods. The results showed that MSL improved the survival rates of *D. melanogaster*, with no toxic effect on the eclosion rate and locomotive capacity. *In vivo*, MSL exhibited a dose-dependent reduction in Nitric oxide (NO) levels (55%), depletion of LPO and H_2O_2 levels across all the groups compared to the control. MSL did not cause any form of alteration in the antioxidant defense system (GST, CAT and TSH and GSH levels) and maintained GST activity while improving GSH and total thiol levels. In conclusion, MSL possesses anti-inflammatory, and antioxidative properties, and is safe in *D. melanogaster*. These observations validate the safety of the fruits of *Sarcocephalus latifolius* used in traditional medicine.

Keywords: *Sarcocephalus latifolius*, Fruit flies, Tumours, Antioxidants, Lipid peroxidation (LPO)

Introduction

The use of medicinal plants for healing is as old as humanity itself, and this dates back to the primeval days,[1] establishing a strong connection between man and nature.[2] Natural medicine represents divine gifts from mother nature present as ready supplements and alternatives in the management of diseases and for the general well-being of man.[3] Over time, man has adapted to the use of certain plant parts including leaves, stems, roots, and fruits as drugs for effective therapy. The only challenge in the use of medicinal plants was the paucity of information on scientific proof of their active ingredients, and the optimal dosage needed to give the desired therapeutic effect.

According to the World Health Organization, we have a higher percentage of people dependent on traditional remedies, from the developing countries of the world (approximately 80%), encompassing the use of extracts from plants for their primary healthcare.[4] This implies that an estimated high number of the world's population is still dependent on plants as sources of drugs[5] and is the most used species for the production of drugs, healthcare products, and novel discoveries in medicine.[3] There is, therefore, increasing use of plant extracts, which are generally regarded as nontoxic, available, cheap, and a socially satisfactory form of healthcare trusted by many people as an alternative to artificial or laboratory-synthesized drugs.[4,6] Notwithstanding plant extracts' effectiveness, significant indications suggest that they can elicit cytotoxicity. Therefore, it becomes important to evaluate or investigate and establish that the extracts used in folk remedies are safe. [7]

Sarcocephalus latifolius (SL) was formerly known as *Nauclea latifolia*, of the family Rubiaceae. The name *Sarcocephalus latifolius* (SL) was derived from the Greek word "*sarco"* (fleshy) and *cephalus* (headed) about the flowers. It is a multi-stemmed tree growing up to 12 m high, and the leaves are usually green.[3,8]

Other names of *Sarcocephalus latifolius* in English are Pin cushion tree, African peach, and Guinea peach and in Nigeria, it is known as 'ubuluinu' or 'Nbitinu' in Ibo, 'Tafashiya', 'Tashiyaigia' or 'Marga' in Hausa, while the Yorubas call it "Egbesi" or "Ogbesi".[3,9] It is an androgynous tree that blossoms from April-June and July-September. This shrub is common in the moist tropical rain or savannah forests of both Central and West Africa. The discrete fruits are bonded into a plump form with a distinctive rutted surface. The tiny seeds are rooted in a pinkish fleshy skin with a strawberry aroma. Fruits are usually fleshy and edible, the stalks and leaves serve as livestock feed, the flowers and pollen provide nectar and pollen to bees respectively, with a woody bark that is termite-resistant, containing tannins used for dyeing.[8]

S. latifolius has been reported to be of great use in the management of various diseases due to its possession of a wide range of medicinal properties. Different parts of the plant including the fruits, leaves, barks, and roots, are used in different traditional setting for the treatment of aches, pyrexia, infection, malaria, diabetes, high blood pressure, and

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Citation: Imah-Harry JU, Abolaji AO, And Olorunsogo OO. Safety Assessment of the Crude Methanol Extract of *Sarcocephalus latifolius* (Sm.) E.A. Bruce Fruits in *Drosophila melanogaster*. Trop J Nat Prod Res. 2025; 9(7): 3279 – 3280 https://doi.org/10.26538/tjnpr/v9i7.55

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

some CNS complications like epilepsy.[10,11] the plant has been reported to have anticancer,[12] anti-inflammatory,[13] antioxidant,[9] antimicrobial, anticonvulsant, and sedative properties. Sound evidence has been provided via a blend of bioassay-guided extraction and standard phytochemical investigations have linked the antiplasmodial, antibacterial, analgesic, and other actions of the plant to the presence of secondary metabolites such as alkaloids, flavonoids, cardiac glycosides, saponins, tannins in several parts of the plant. There is hardly any part of *Sarcocephalus latifolius* that has not been found therapeutically valuable.[14]

In this study, the possible noxiousness of the crude methanol extract of *S. latifolius* (Sm.) E.A. Bruce fruits (MSL) in *Drosophila melanogaster* (fruit fly) was investigated. A range of natural delicacies in the urban population of developing countries contain ingredients from fruits, leaves and even seeds of many plants. Micro or macronutrients such as vitamins, minerals, soluble sugars, dietary fibers, and edible plant derived-polysaccharides, are known to be easily accessed in the body by the increase of fruit intake.[15] *Drosophila melanogaster* as a model raises little or no ethical concern and serves as a unique, inexpensive, and powerful model organism useful in studies investigating the in-depth of chemical and natural products, via their pharmacological and toxicological proprerties.[16] The fly also shares several basic parameters, such as the biological, neurological and physiological resemblances evident in humans. Scientific documentation has also proven that about 75% of humanoid disease-causing genes have an efficient homolog in *D. melanogaster*.[17] It has been established that a lot of people depend on the use of *S. latifolius* as a medicinal plant but the ability of some of the plants and their metabolites to cause undesirable effects, led to the investigation on the effects of crude methanol extract of *S. latifolius* fruits (MSL), on parameters such as survival, locomotor, behavioral, and neurological activity, and on antioxidant defenses in *D. melanogaster*.

Materials and Methods

Plant sample collection

The ripened fruits of *S. latifolius* were arbitrarily sourced from diverse twigs of the shrub developing in the forestry areas around Errua village, Iddo LGA, Oyo state, Nigeria, in December, 2021. The samples were conveyed on the same day to the Drosophila Laboratory, Biochemistry Department, University of Ibadan, Nigeria in airtight polyethylene bags. Herbarium samples of SL whole plants were reaped and dispatched to the Forestry Research Institute of Nigeria (FRIN) and the voucher number FHI 110092 was issued. After authentication, sample specimen was dropped in Pharmacognosy Department, University of Ibadan's herbarium.

Plant Extraction

The plant samples were washed, pulverized with a porcelain mortar and pestle, and air-dried in a clean airy space. The powdered sample was stored under nitrogen gas in an airtight glass container. 100g of the pulverized sample was weighed into glass containers, 250 mL of double-distilled methanol (100%) was added and shaken with an adjustable vibrator shaker at 25^0C. The test medium was left to stand overnight at 25^0C, and shaken intermittently every 3 hours for 72 hours. The extract was thereafter filtered, and the filtrate was concerted to a brown slurry using a Rotovap machine. The concentrated methanol extract of *Sarcocephalus latifolius* fruits, (MSL) was stored at 4 ^{0}C until further analysis. Methanol was the extraction solvent of choice because being a polar solvent it has the capacity to excerpt a extensive variety of bioactive compounds in plants, with the ability to extract at a relatively low temperature, thus minimizing degradation while preserving the integrity of the sensitive bioactive compounds.

Determination of Plant Extract Yield

The yield of the methanol extract of *S. latifolius* (Sm.) E.A. Bruce was considered founded on the dry mass of the powdered plant sample using the formula below:

Yield (MSL g/100g of dry plant material) = (W1 × 100)/W2

Where;

W1 and W2 are the weight of the MSL extract after solvent evaporation and the weight of the dry plant material, respectively.

Drosophila melanogaster culture

The fruit flies (*D. melanogaster*) used for the study was supplied by the Department of Biochemistry and Molecular Biology, Federal University of Santa Maria, Brazil, from the laboratory of Prof. JBT Rocha. The flies were sourced from National Species Stock Center (Bowling Green, OH, USA), as "wild type (Harwich strain)". In Nigeria, the rearing and maintenance of the flies were at the designated Laboratory, where the flies were maintained in cornmeal medium composed of powdered milk (1% w/v), agar (1% w/v), brewer's yeast (1% w/v), sucrose (2% w/v) and Nipagin (0.08% w/v). This medium was maintained at continuous temperature "(22–24^{0}C)", and moistness "(60–70% relative humidity)" under twelve-hour dim/bright phase, all through the period of the study. All experiments were performed using both sexes at random.

Treatment of D. melanogaster with MSL

For effective exposure of the fruit flies to the extract, both genders, (age: one to three days old) of *D. melanogaster* wild-type (Harwich strain) were separated into six groups of fifty flies each and exposed to varying doses (very low to high) of crude methanol extract of *S. latifolius* fruits (MSL), as shown below:

Group 1: Wild-type control (distilled water alone)
Group 2: MSL 0.1 mg/kg diet
Group 3: MSL 0.2 mg/kg diet
Group 4: MSL 0.5 mg/kg diet
Group 5: MSL 1 mg/kg diet
Group 6: MSL 10 mg/kg diet

The flies in all groups were exposed to the treatments in the MSL diet, for a period of 28 days. Thereafter, the following parameters were evaluated: behavioural (acetylcholinesterase, ACT and negative geotaxis, NG), inflammation [nitric oxide ("nitrate and nitrite", NO)], lipid peroxidation (LPO), oxidative stress, and antioxidant status ("hydrogen peroxide", H_2O_2, "total thiol" TTC, "glutathione content", GSH, catalase, CA and "Glutathione-S-transferase" GST) and eclosion rate, ER.

Emergence, locomotor capacity, and survival rate of flies

Determination of eclosion rate (progeny development) of *D. melanogaster* offspring after their periodic introduction to the various MSL doses. The locomotive capability was also assessed by means of the "negative geotaxis" behavior as described by [18] and [19] with slight modifications, while the survival rate was assessed via a day-to-day record of fruit flies' deaths, and the information were evaluated.[20,21]

In vivo assays

Processing and sample preparation for biochemical assays

All the flies in the allotted groups, including the control group were anaesthetized using CO_2 gas. They were weighed and homogenized in 0.1 M potassium phosphate buffer, pH 7.4 [1:10 (flies/volume (µL)], for the biochemical assays. Centrifugation of the homogenized flies were achieved at 4,000× g for ten minutes at 4^0C in a Thermo Scientific Sorvall Legend Micro 17R centrifuge. Using Eppendorf tubes for storage of the aliquots, the supernatants were separated from the pellets and preserved in the freezer at -20^0C until ready for use. Thereafter, the assessment of catalase activity (CA), GST activities, GSH, T-SH, acetylcholinesterase (AChE), and H_2O_2 levels as biochemical tests, were performed using aliquots of the supernatants. For apiece of the 5 duplicates of control and treated flies, all the tests were duplicated.

Biochemical assays

Determination of protein concentration

The valuation of protein levels in samples was assessed according to the method of [22], using bovine serum albumin (BSA) as the standard.

Determination of total thiol content (TTC)
The TTC in all the groups was evaluated following the technique previously described by [23] A solution containing 510 µL of phosphate buffer (0.1 M, pH 7.4), 20 µL MSL, 35 µL of 1 mM Ellman's reagent (5, 5'-dithiobis-(2-nitrobenzoic acid) and 35 µL of distilled H_20 (DW) were mixed. To incubate for thirty minutes before taking the absorbance reading at 412nm, using a spectrophotometer (UNISPEC 23D, UNISCOPE, England), this combination was set aside. Finally, the TTC was computed and expressed in µmol/mg protein, with GSH used as standard.

Determination of glutathione S-transferase (GST) activity
The activity of GST following treatment with MSL was quantified in agreement with the procedures of [24] This method is an enzymatic assay that uses a substrate and enzyme. The substrate used for the reaction was "1-chloro-2,4-dinitrobenzene (CDNB)". With other constituents of the analyzing mixture including 10.5 µL distilled water, 500 µL CDNB, 20 µL MSL (1:5 dilution), and 270 µL of a solution consisting of phosphate buffer, pH 7.0 and EDTA. The reaction mixture was observed for 5 min and the OD readings taken at 340 nm, at intermissions of ten seconds using a spectrophotometer (UNISPEC 23D, UNISCOPE, England). GST results using a molar extinction coefficient of 9.6 mM^{-1} cm^{-1}, were expressed in µmol/min/mg protein

Evaluation of glutathione (GSH) content
The GSH concentration was determined spectrophotometrically using Ellman's reagent (DTNB) following the method designated by [25] The upper layer was precipitated by mixing with a 4% solution of sulfosalicylic acid in a 1:1 ratio. Test samples of MSL were incubated at 4^0C for an hour, then centrifuged at 5000× g for 10 min at 4^0C. The reaction mixture consists of 100 µL of DTNB, 550 µL of phosphate buffer (0.1 M), and 100 µL of the supernatant. At a wavelength of 412nm, the absorbance of the reaction mixture was taken with the aid of a spectrophotometer (UNISPEC 23D, UNISCOPE, England). The result was expressed as moles of GSH/gram tissue.

Determination of catalase activity (CA)
Catalase activity was determined by the procedure described by [26] A 100 mL of the reaction mixture was primed by incorporating 50 mL of 0.05 M phosphate buffer (pH 7.4), 194 µL of 30% H_2O_2 and made up to volume with DW. Into a 1 cm quartz cuvette with 10 µL of MSL, as test sample, '19 mM solution of hydrogen peroxide (590 µL) was pipetted. This test combination was quickly mixed by inverting it intermittently, and the reduction in the OD readings, of H_2O_2 ($\varepsilon = 39.4$ mM^{-1} cm^{-1}) at 240 nm, with prompt monitoring at intervals of 2 min, was measured using a UV/visible spectrophotometer (UNISPEC 23D, UNISCOPE, England). The CA was calculated from the absorbance data, and results were stated as µmol of H_2O_2 consumed per minute per milligram (µmol/min/mg) of protein.

Determination of acetylcholinesterase activity (ACT)
The method defined by [27] was adopted for the assessment of the acetylcholinesterase activity. The reaction mixture made up of 20 µL 10 mM DTNB, 20 µL 8 mM acetylthiocholine, 135 µL DW, 5 µL of MSL, and 20 µL of 100 mM potassium phosphate buffer (pH 7.4). The absorbance was measured at 412 nm every 15 seconds for 5 min using a SpectraMax microplate reader (Molecular Devices, USA). The figures generated from this enzyme action assay was hydrolyzed per minute per milligram (µmol/min/mg) protein and valued as µmol of acetylthiocholine.

Evaluation of hydrogen peroxide (H_2O_2) concentration
The H_2O_2 concentration in MSL was estimated by adopting the method of Wolff (1994).[28] The reaction mixture included 10 µL of MSL added to a 590 µL of FOX (ferrous oxidation-xylenol orange) solution containing a mixture of xylenol orange, ammonium ferrous sulfate, sorbitol, H_2SO_4, and distilled water. Then, this was incubated at 25^0C for 30 minutes, and the OD taken at 560 nm. With the aid of a standard curve, data from the assay were extrapolated and results were computed as final values of the H_2O_2 levels, expressed in micromole per milligram (µM/mg) protein.

Determination of nitric oxide (nitrate/nitrite) level (NO)
The NO level in MSL was measured using Griess reaction as described by [29] The method is centered on the conversion of nitrate to nitrite enzymatically. Using a 1:1 ratio, MSL samples were mixed with Griess reagent: 250 µL of MSL and 250 µL of Griess reagent and set aside at 25^0C for twenty minutes. With a spectrophotometer, the absorbance was taken at 550 nm and the quantification of NO concentration was computed by the contrast evaluation of OD results with data obtained from the sodium nitrite standard curve measured at 550 nm.

Assessment of lipid peroxidation
The procedure of [30] was adopted in the estimation of Lipid peroxidation (LPO) in the MSL, by assessing thiobarbituric acid reactive substances (TBARS) production during the reaction. Malondialdehyde (MDA) is a by-product of the LPO of membranous fatty acid and nutrition yields from food reacting with the chromogenic reagent, 2-thiobarbituric acid (TBA). During the estimation of LPO, a pink-colored complex extractable with an organic solvent such as butanol was generated and the absorbance was read at 532 nm. The results were communicated in via the amount of MDA produced per mg of protein.

Statistical analysis
Data from the survival and longevity assays were analysed adopting the Kaplan-Meier method by comparing the data obtained with the log-rank test. Values obtained from the biochemical assay were presented as the Mean ± standard error of the mean (SEM). Mean variances amongst various groups were evaluated by ANOVA, and the Dunnett's post hoc test. Statistical significant difference was established at $p < 0.05$ via the Graph Pad Prism 5.0 software.

Results and Discussion

In the twentieth century, there has been an enormous breakthrough in healthcare, despite this breakthrough, the majority of the world's population in developing countries lack steady access to modern pharmaceuticals. Given this challenge, a treatment option using contemporary medicine is not a likely prospect for these people.[9] As an alternative, they resort to the ingestion of medicinal plants with beneficial and healing attributes, useful in the management of pathological situations. This is a common practice, as products of herbal medicine produced from medicinal plants are used as dietary or food supplements. They are sold as fresh or dried plants and extracts by traditional medical practitioners, tablets, capsules, powders, teas, etc. in nutraceuticals.[31] This fact contributes to the incidence of intoxication, with the likely presence of secondary metabolites with toxic properties. These toxic secondary metabolites include phytochemicals such as alkaloids, terpenoids, tannins, and glycosides.[32-33] Evidence from *in vitro* and *in vivo* studies indicates that these phytochemicals cause toxicity in humans, aside from exerting a variety of pharmacological actions such as lifespan elongation, delay of the aging process, etc. The toxic effect of plants has been documented to be by various mechanisms hence making it very necessary to investigate the potential toxicity of plant extracts commonly utilized in traditional medical practice.[34]

In this regard, about a century ago, *D. melanogaster* (fruit fly) was introduced as a decisive model and an outstanding animal model for studies in genetics, biochemistry, cell biology, and evolution, just to mention a few. More recently, it was also described as a model for toxicological screening or the study of various potent compounds of both natural and synthetic origin.[35] Given these attributes, the fruit flies, therefore, befits a striking model for investigating plant biological properties. This is because the candidate plant extracts, solvent fractions, or compounds sequestered from it can be introduced in diverse developing stages of the fly, merely by mixing it with the fly's medium. Additionally, the Drosophila genome comprises homologs of approximately 70-77% of the disease-related loci in people and over 85% of genes linked with a mental incapacity.[35] Another hypothetical relevance is the fact that the fly shares numerous rudimentary natural, biochemical, neurological, and physiological resemblances with humans.[17]

In this study, we examined the possible noxiousness of crude methanol extract of *S. latifolius* (Sm.) E.A. Bruce fruits (MSL) in fruit flies. This was achieved by exposing the flies to stated dose regimen of MSL to

probe the dose-response correlation on the survival, behavioral, neurotoxic effect, and antioxidant cellular defense system (ACDS) as well as enzymatic CAT and GST activities, and non-enzymatic glutathione (GSH) levels that act on nitrogen and reactive oxygen species subsequent from pathological or physiological processes in Drosophila melanogaster.[36]

Effects of MSL on the survival rate of D. melanogaster

The locomotive performance of exposed flies to the control and MSL diets, ad libitum, at varying concentrations is presented in Figure 1. This is a time dependent monitoring activity for the first seven days, using a length of 6cm indicated in the vials containing the sorted fruit flies.

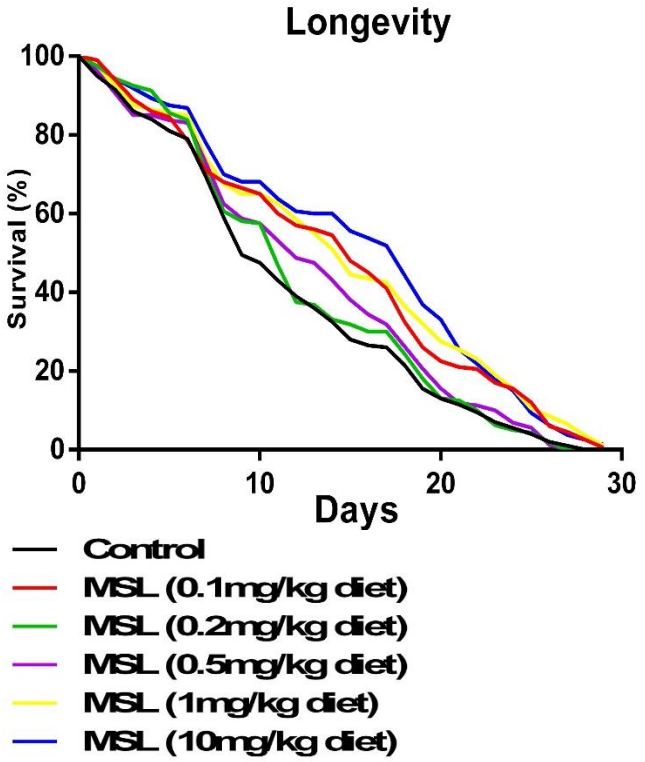

Figure 1: Effects of MSL on survival rate. The maximum lifespan in each group represents the percentage of surviving flies

Overall, the capacity of flies to climb over this length in 6 seconds, emerge, and have an increased rate of survival after exposure to MSL are good indicators of the safety of MSL. It could therefore be claimed that MSL does not affect the embryo inside the egg and does not cause any delay in larvae emergence from the eggs. A further review of the results revealed that even with an increased dose of MSL at 10 mg/kg, the rate of larvae transforming into pupae and pupa to adult was comparable to that of the control group. This infers that the accumulation of MSL in the fly had no deleterious effect on the larvae, thus there was no substantial alteration in the existence potential of the larvae and the rate of larva transformation to pupae in both control and treatment groups after 28 days.

Effect of MSL exposure on antioxidant status of D. melanogaster

The effects of MSL on certain antioxidant parameters in *D. melanogaster* is revealed in figure 2. The results showed that the exposure of the fruit flies to MSL for 28 days had no significant alteration in the TTC (Figure 2A), GSH (Figure 2B), GST (Figure 2C), and catalase (Figure 2D). However, the 10 mg/kg MSL diet significantly increased GSH level (1.85 folds), when matched with the control group ($P < 0.05$). This designates that MSL confers protective roles by maintaining both antioxidants systems in the flies, whether enzymatic or non-enzymatic. Thiols, which act as reducing agents to preserve cellular redox homeostasis, are compounds enriched with cysteine, having a carbon-bound sulfhydryl group.[37] TSH are comprised mainly of GSH and some other thiol-containing compounds in tissues. Furthermore, reflected by Glutathione is the redox state of –SH groups, vital for the action of many proteins. Glutathione-S-transferase (grouped as a family of phase II detoxification enzymes), confers protection to tissues by minimizing oxidative damage via the conjugation of glutathione to electrophilic centers of exogenous and endogenous electrophiles (toxic products of Phase I detoxification). They achieve this by converting them to less harmful products.[19] GSH acts as a portion of the crucial measure of the antioxidant cellular defense system in existing creatures, and in combination with GSH-dependent enzymes, it aids in the inhibition of the proliferation of free radical chain reactions and clean up toxic reactive oxygen and nitrogen species. The fact that *S. latifolius* (Sm.) E.A. Bruce maintained GST activity while improving GSH and total thiol levels further contributing to the maintenance of redox balance in *D. melanogaster* which may be through the activation and triggering of antioxidant responsive element (ARE) including Nrf-2.[38] Catalase, an enzyme containing heme,[23] catalyzes the dismutation or transformation of H_2O_2, a potentially toxic reactive oxygen species (ROS) to O_2 and H_2O, leading to a reduction in oxidative stress-enhanced toxicity and ROS-mediated damage. The results of this investigation show that no substantial variance was experiential in the catalase activity (CA) of the MSL-exposed flies and control group. This implies that the CA was sufficient to cause a significant decrease in the level of H_2O_2 accumulation in the fruit flies as MSL inhibited the Fenton reaction observed with enhanced production of OH^- radicals and/or other ROS as by-products. Therefore, the ability of MSL to induce inhibition of H_2O_2 accumulation indicates that *Sarcocephalus latifolius* fruits might have both antioxidative and free radical rummaging activities.

Effect of MSL exposure on oxidative stress markers in D. melanogaster

Figure 3 shows the levels of accumulation of NO (nitrate and nitrite), H_2O_2, and rate of lipid peroxidation in *D. melanogaster* after 28 days of exposure to MSL. A decline of almost 55% reduction in the group fed with a 10 mg/kg MSL diet, aligning in the dose-dependent style was observed in NO levels, when matched with the control group ($P < 0.05$) (Figure 3A). A depletion in the H_2O_2 levels and LPO was also observed (Figure 3B and 3C, respectively) and a similar trend was observed in the flies treated with a 1 mg/kg MSL diet with each parameter showing a significant reduction of about 25% when likened with the control group ($p < 0.05$). NO is implicated in the pathogenesis of several diseases. It is considered a pro-inflammatory metabolite owing to its potential to act in response with superoxide anion and other free radicals. This potential causes toxicity due to the production of a more deleterious nitrite anion and other reactive by-products.[18] As a diffusible and transitory free radical gas, NO can form stable metabolites nitrate and nitrite by rapid recombination, while it serves as a common biomarker in inflammatory disorders arising from tissue damage.[39] Reduced levels of NO in the flies treated with varying concentrations of MSL suggest that the minimal level of NO that is essential to perform physiological functions was reinstated in the flies. Therefore, *S. latifolius* (Sm.) E.A. Bruce possesses anti-inflammatory properties. Lipid peroxidation (LPO) represents a kind of oxidative damage or degradation that originates from diverse ROS radical species or by the catalytic degradation of tissue lipid hydroperoxides.[40] The termination of this peroxidative damage yields several dangerous aldehyde products such as MDA and 4-hydroxynonenal (4-HNE), despite the structural and functional defect of the phospholipid bilayer of membranes. Furthermore, proteins or DNA may undergo secondary damage, which may arise from the products of peroxidative damage.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

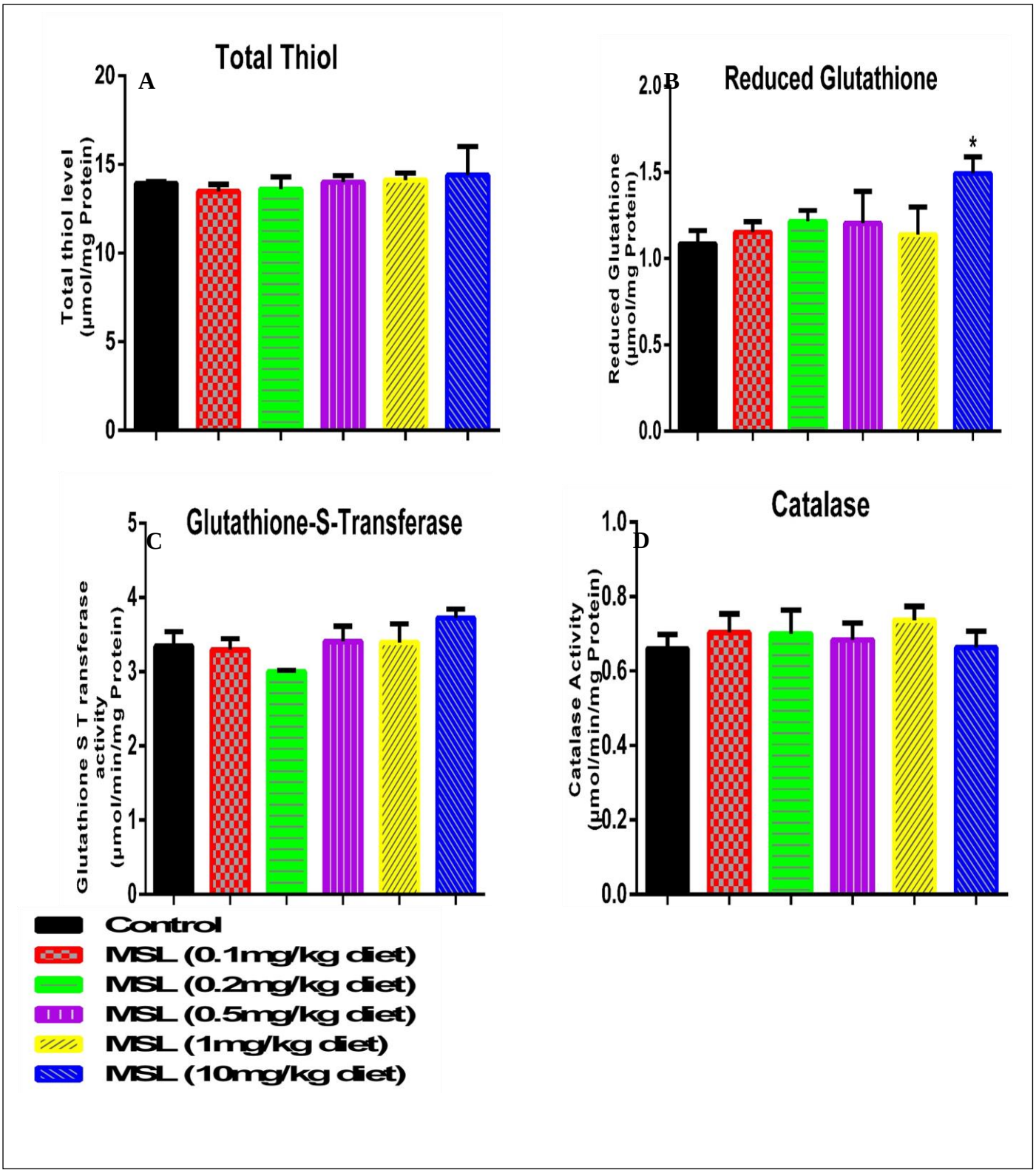

Figure 2: The effect of MSL on antioxidant parameters in *D. melanogaster*. **(A)** Total thiol, **(B)** Glutathione, **(C)** Glutathione S-Transferase and **(D)** Catalase. Data are presented as Mean ± SEM of 40 flies/vial with 5 replicates per treatment group. The asterisk (*) indicates significant differences when compared with the control group at $p < 0.05$

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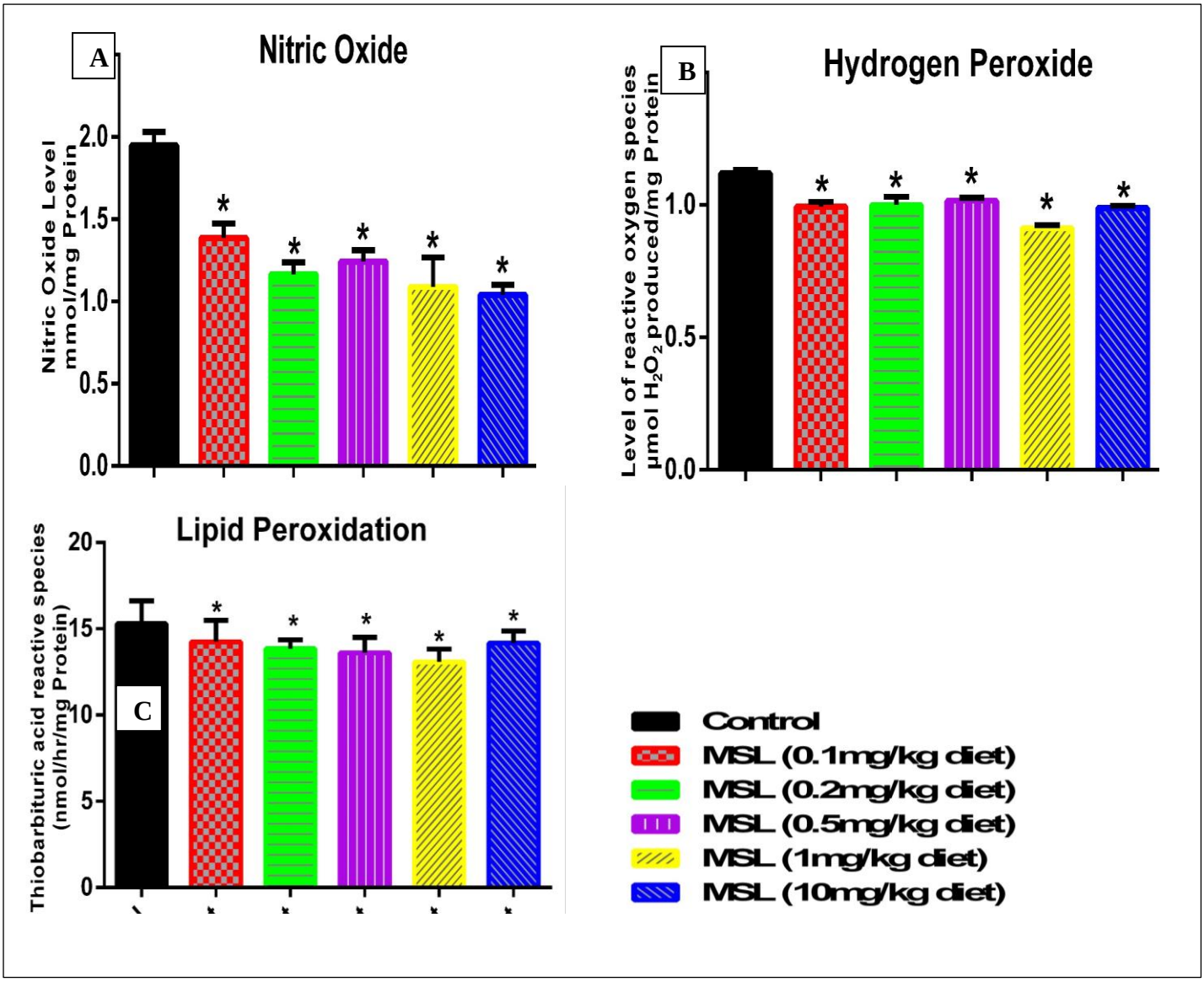

Figure 3: Effect of MSL on markers of oxidative stress in *D. melanogaster*. **(A)** Nitric oxide, **(B)** Hydrogen peroxide and **(C)** Thiobarbituric reactive species. Values are expressed as Mean ± Standard Error of Mean, (n = 5). The asterisk (*) indicates significant differences when compared with the control group at $p < 0.05$.

The present study confirmed that MSL based treatment of the flies, caused a substantial decline in the level of TBARS, the product of LPO in the flies when likened to the flies in the control. It can therefore be proposed that the protective effect of MSL may probably be due to the antioxidant activity of MSL, which reduces oxidative damage by preventing the formation of free radicals and thus suppresses LPO.

Effect of MSL on eclosion rate, locomotive capacity, and ACT in D. melanogaster

Afterwards exposing the flies to MSL for the first 7 days, the count of the total offspring was taken only for the fully formed adult flies, daily. As illustrated in Figure 4A, there was no noteworthy or noticeable variance in the degree of adult emergence (eclosion) in the control and the treatment groups (0.1, 0.2, 1, and 10 mg/kg MSL diet). Also, the locomotive capacity of *D. melanogaster* (Figure 4B), showed no significant difference as both the control and treatment groups had the same climbing rate. It was observed that all the flies were able to climb the walls of the vials easily and freely at all concentrations of MSL, thus indicating the non-toxic effect of MSL on the flies. A parallel style was seen in the activity of acetylcholinesterase, where there was no noteworthy alteration in the activity of acetylcholinesterase (Figure 4C) in the control and the treatment groups after 7 days of exposure. This also indicates that MSL maintained the basal activity of the enzyme needed for optimum nervous coordination.

Acetylcholinesterase (AChE), a vital constituent of the cholinergic organization, catalyzes the hydrolysis of ACh, to choline and acetate thus impeding cholinergic neurotransmission,[41] playing an important role in locomotion and the regulation of motor function. This fact has been authenticated in eukaryotic (vertebrate) systems, but the accurate result in fruit flies has not been scientifically clarified.[42] While some scientific research has associated AChE inhibition with oxidative stress, both an upsurge and a reduction in AChE may have harmful effects or significance in *D. melanogaster*.[43,44] Our results revealed that MSL moderated the activity of AChE, which was evident in the improved locomotor capacity of the fruit flies.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

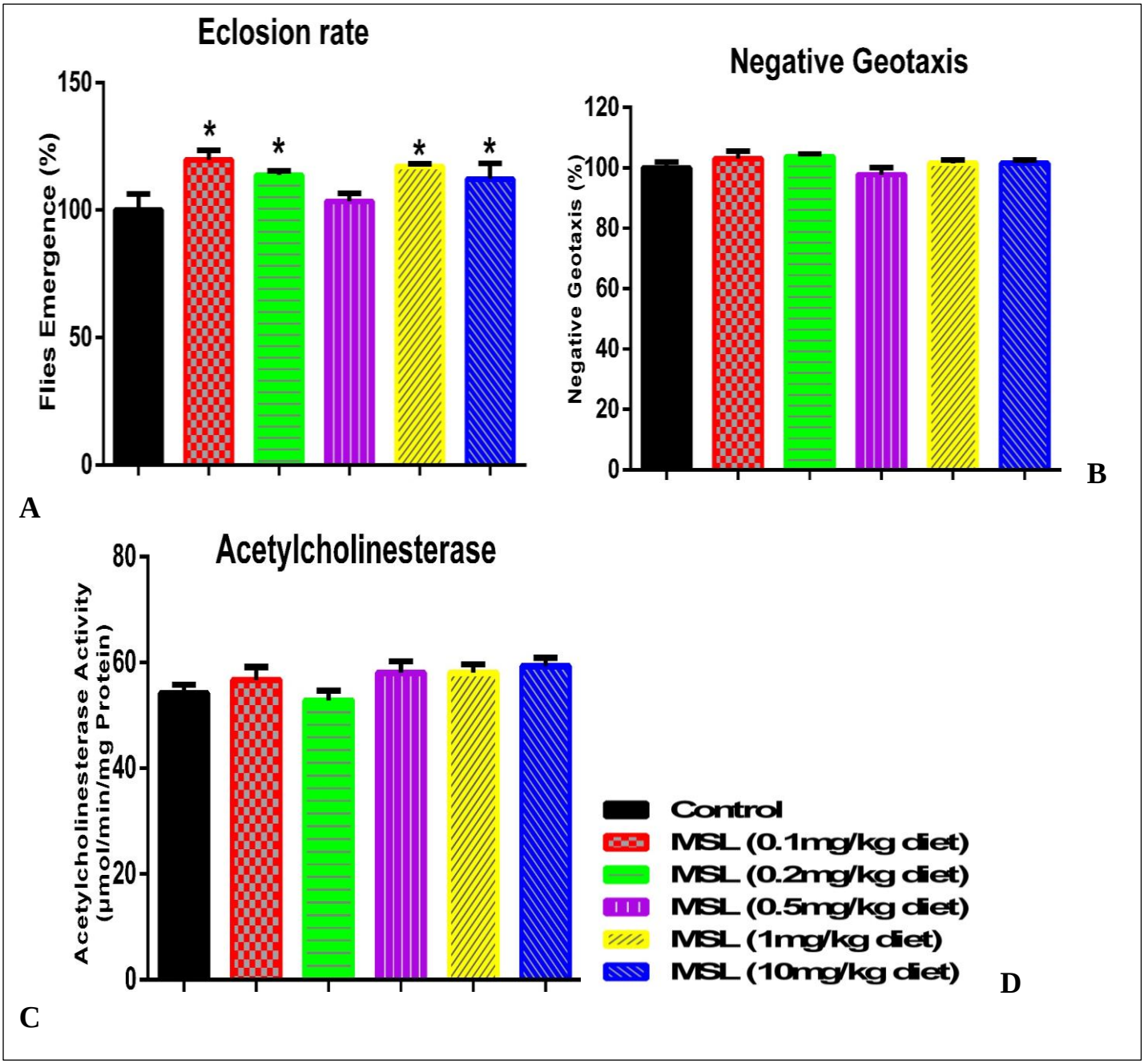

Figure 4: Effects of MSL on (A) eclosion rate (B) locomotive capacity (Negative geotaxis) and (C) acetylcholinesterase activity in *D. melanogaster*. Values are expressed as Mean ± Standard Error of Mean, (n = 5). The asterisk (*) indicates significant differences when compared with the control group at $p < 0.05$.

Conclusion

The outcome from the present study indicate that the crude methanol extract of *Sarcocephalus latifolius* (Sm.) E.A. Bruce fruits (MSL) is safe in *D. melanogaster* (fruit fly). The oral administration of fruit flies to MSL caused no mortality, locomotive deficit, oxidative damage, neither did it cause any form of alterations in the antioxidant system nor discrepancies in acetylcholinesterase activity. These findings also buttress the antioxidant, anti-inflammatory and possible neuroprotective effects of the plant. Therefore, these observations validate the safety of *Sarcocephalus latifolius* fruits used in folklore remedy in the management of several ailments.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

We acknowledge Dr. Adeola Adedera, who was always there to make sure the Drosophila laboratory, University of Ibadan and all we needed to work was always made available to us during the course of the study.

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