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Effect of Aqueous Extract of *Momordica charantia* on Survival, Locomotive Behaviour and Antioxidant Status of *Drosophila melanogaster*

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ABSTRACT

Momordica charantia, commonly known as bitter gourd, is a therapeutic plant popularly known for its antidiabetic potential in folklore medicine. This study investigated the effect of aqueous leaf extract of *M. charantia* (AMC) on survival rate, locomotive behaviour and antioxidant response in *Drosophila melanogaster*. Adult flies were fed with different concentrations of AMC (0-100 mg/mL) for 12 days, and their locomotive behaviour and whole-body antioxidant status were assessed at 0, 2, 4 and 8 mg/mL of AMC. Results showed a significant decrease ($p < 0.05$) in the survival rate and locomotive behaviour of flies at 8, 16 and 50 mg/mL of AMC compared to the control. There was no significant difference in malondialdehyde content, GSH level and SOD activity of flies exposed to 2, 4, and 8 mg/mL of AMC compared to the control group. Glutathione-s-transferase activity at 8 mg/mL of AMC increased significantly ($p < 0.05$) when compared to the control group. Acetylcholinesterase activity also increased in a dose-dependent manner with a significant increase at 4 and 8 mg/mL of AMC. The evidence from this study suggests that low to moderate doses of aqueous extract of *Momordica charantia* slightly improved survival rate of flies. It also increased the activities of acetylcholinesterase and antioxidant enzymes.

Keywords: *M. charantia*, Survival rate, *D. melanogaster*, Locomotive Behaviour, Antioxidant.

Introduction

Plants rich in phytochemicals and phytonutrients have played an essential role in the overall health and wellness of several individuals in the world for ages.[1,2] In developing countries, they still serve as the primary source of health care among some communities while in some developed countries, they serve as alternative and complementary medicines.[3,4] *Momordica charantia* L. is one of the numerous therapeutic plants known today. It is used in ethnomedicine to manage and treat ulcers, jaundice, type 2 diabetes, viral and bacterial diseases.[5] It is commonly called bitter gourd or bitter lemon because of its different parts' bitter taste.[5] Locally, it is known as "Ejirin" among the Yorubas, Alo-ose among the Igbos and Kakayi among the Hausas in Nigeria [6]. Apart from its therapeutic value, it is also known for its nutritional properties. Unlike most bitter vegetables, the bitter taste of the fruit of *M. charantia* is considered suitable for consumption. It is often added to other vegetables and food to give a bitter taste and flavour.[5,7] The whole herb can also be boiled and taken as a tea. The leaves, fruits, stems, and seeds contain proteins, carbohydrates, lipids, minerals and vitamins.[5,8]

Drosophila melanogaster, commonly called fruit fly, is used as a model organism for studying different human diseases.[9] About 75% of the genome of *D. melanogaster* are functionally related to that of humans. The fruit fly also produces a large number of offspring within the shortest time. It is small, not costly to maintain in the laboratory and has a short lifespan, making it a viable model organism.[9–11] Despite the extensive use of *M. charantia* in folklore medicine and the various scientific studies validating its therapeutic potential, its toxicity has not been fully explored, particularly in *D. melanogaster*. This study examined the effect of aqueous extract of *M. charantia* (AMC) on survival, locomotive behaviour and antioxidant status in *D. melanogaster*.

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Materials and Methods

Preparation of plant material

M. charantia vines were harvested fresh, in October 2018, from local farms in Ota, Ogun State, Nigeria, and the leaves were separated. A botanist, Dr. J. O. Popoola, at the Department of Biological Sciences, Covenant University, Ota Ogun State, identified the plant and voucher specimen (with number; FHI 112033) was kept at the Forestry Research Institute of Nigeria. The leaves were air-dried, ground to powder and extracted with distilled water (400 g in 4 L) for 72 h. The resulting extract was evaporated to dryness under vacuum in a rotary evaporator at 55°C.

Strain and culture of D. melanogaster

Harwich strain (wild-type) of *D. melanogaster* was raised at the Department of Biochemistry, Covenant University Ota. Male and female flies were fed brewer's yeast paste on an apple agar medium in a standard egg collecting cage. Eggs (6 hours old) were collected, rinsed in sterile 1x phosphate buffer saline (PBS) and used for further experiment.

Survival assay

D. melanogaster eggs were raised on a regular diet (containing 10 g of agar powder, 15 g of yeast, 50 g of sugar, 50 g of semolina and 1.2 g of paraben) and different concentrations of AMC (0, 1, 2, 4, 16, 32, 50

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and 100 mg/mL). The developmental process was monitored until moulting into the adult stage. Adult flies (1-3 days old), 50 per vial, enclosed from embryos raised on different concentration of AMC were further exposed to regular diet and different concentrations (0, 1, 2, 4, 16, 32, 50 and 100 mg/mL) of AMC for 12 days. The mortality and survival rates of the flies were monitored each day for 12 days. The diet mixed with different concentration of AMC was replaced every 72 h. The results were presented as the percentage of life flies.[12]

Negative geotaxis assay

Flies treated with different concentrations of AMC were examined for their locomotive performance, using previously described methods.[12,13] Data generated were analyzed and represented as a percentage of the mean number of flies above the 6 cm mark.

Whole fly homogenate preparation

After exposure of adult flies to different concentrations of AMC for 12 days, 10 flies were slightly made inactive under mild ice. The flies were homogenized in homogenizing buffer containing 1 mM EDTA, 0.25 M sucrose and 10 mM HEPES NaOH pH 7.4. The homogenate was centrifuged at 4000 rpm for 10 min at 4^{o}C in a refrigerated centrifuge. After centrifugation, the supernatant was stored at -20^{o}C until the time of use.[14]

Assessment of antioxidant status and acetylcholinesterase activity

The extent of lipid peroxidation was assayed spectrophotometrically by determining malondialdehyde (MDA) concentration in the fly homogenate.[15] Glutathione-s-transferase (GST) activity was evaluated based on the method of Habig *et al*.[16] while superoxide dismutase (SOD) activity was evaluated based on pyrogallol autoxidation method described previously.[17] Glutathione level in fly homogenates was estimated according to the method described by Moron *et al*.[18] Acetylcholinesterase activity was assessed in fly homogenate based on the hydrolysis of acetylcholine iodide as reported by Ellman *et al*.[19]

Total protein determination

The total protein level in the fly homogenates was determined by Lowry's method using bovine serum albumin as a standard.[20]

Statistical analysis

Data generated in this study were subjected to one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (version 20.0, SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to compare the level of heterogeneity among groups at $p < 0.05$. The results are presented as mean ± SEM of three biological replicates

Results and Discussion

M. charantia is a medicinal plant with both therapeutic and nutritional properties. Despite its wide use in folklore medicine, there are still growing concerns about its toxic effect. Also, little is known on how the plant extract affects the survival rate, locomotive behaviour and antioxidant response in *D. melanogaster*. This current study found that AMC caused the death of all flies at 100 mg/mL. There was no significant difference in the survival rate of adult flies exposed to 1, 2 and 4 mg/ml of AMC when compared to the control. However, the survival rate of flies reduced significant reduction ($p < 0.05$) at 8, 16 and 50 mg/mL of AMC when compared to the control (Figure 1A and 1B). Findings from this study also showed that the locomotive behaviour of flies was significantly ($p < 0.05$) reduced when compared to the control, at 4, 8, 16 and 50 mg/mL of AMC (Figure 1C). Based on the result of the survival rate and negative geotaxis assay, AMC concentrations of 2, 4 and 8 mg/ml were chosen to evaluate the antioxidant status and acetylcholinesterase activity of *D. melanogaster* that were fed low to moderate doses of AMC. Findings from the antioxidant evaluation showed that there was no significant difference ($p < 0.05$) in MDA content of flies exposed to 2, 4, and 8 mg/mL of AMC when compared to the control group (Table 1). There was also no significant difference in the GSH content of the treated groups when compared to the control (Table 1). Glutathione-s-transferase activity however increased significantly ($p < 0.05$) in a dose-dependent manner among the treated groups when compared to the control group (Table 1). The increase was significant at 8 mg/ml of AMC ($p < 0.05$). Superoxide dismutase and acetylcholinesterase activities also increased in a dose-dependent manner. However, for acetylcholinesterase activity, the increase was significant at 4 and 8 mg/ml of AMC (Figure 2).

The fact that AMC, at 100 mg/ml, caused 100% mortality of flies even before the end of the experimental period indicates a lethal dose which affects the survival and life span of adult flies. Although AMC at 50 mg/ml did not lead to 100% mortality of adult flies, it significantly reduced the survival rate and locomotive performance of the flies. Based on these findings, malondialdehyde content and antioxidant status of flies exposed to 2, 4 and 8 mg/ml of AMC were assessed.

Malondialdehyde is one of the secondary products of lipid peroxidation and a prominent biomarker for assessing the extent at which reactive oxygen species react with lipids. In most biological systems, the effects of reactive oxygen species and other oxidants are neutralized by enzymatic and non-enzymatic antioxidants.[21] Glutathione-s-transferase and superoxide dismutase are two important enzymatic antioxidants that help to reduce the harmful effects of reactive oxygen species and their conjugated products.[22,23] The fact that MDA levels among the flies exposed to 2, 4 and 8 mg/mL of AMC compared well with that of the control suggest oxidant production within the ability of endogenous antioxidants defence system. Also, flies exposed to AMC had a dose-dependent increase in superoxide dismutase and glutathione-s-transferase activities. However, for glutathione-s-transferase activity, the increase was significant at 8 mg/mL of AMC. This suggests that AMC induces synthesis of the antioxidant enzyme, glutathione-s-transferase, and thus have antioxidant activity in *D. melanogaster*. Also, the fact that AMC increased acetylcholinesterase activity in a dose-dependent manner suggests that at low to moderate doses, AMC could be an excellent activator of acetylcholinesterase. Acetylcholinesterase is involved in the metabolism of acetylcholine, an important neurotransmitter that plays a crucial role in the nervous system.[24] Inhibition of acetylcholinesterase causes acetylcholine levels to increase and leads to muscular weakness and paralysis. Though a decrease in acetylcholinesterase activity is often associated with locomotive defects [12,13] in *D. melanogaster*, there are contradictory reports about this. One study showed that, though hydroalcoholic extract of *Croton Campestris* affected the locomotive performance of flies at 0.1- 50 mg/mL, it did not cause any observable difference in acetylcholinesterase activity.[25] Findings from this study corroborate previous studies that showed that medicinal plants are toxic at moderate to high doses in *D. melanogaster*.[13,26,27] The findings observed in this study also mirror those of the previous studies that have examined the toxic effect of *M. charantia* studies in other models. A recent study showed that the LD_{50} of seed extract of *M. charantia* was 50 µg/mL. In comparison, the fruit extract did not cause any toxic effect at a dose of 200 µg/mL in Zebrafish embryos.[28] Another study showed that *M. charantia*, at a dose of 1000 mg/kg body weight was not toxic to male and female Sprague-Dawley rats.[29]

Conclusion

The evidence from this study suggests that low to moderate doses of aqueous extract of *Momordica charantia* slightly improved survival rate of flies. It also increased the activities of acetylcholinesterase and antioxidant enzymes.

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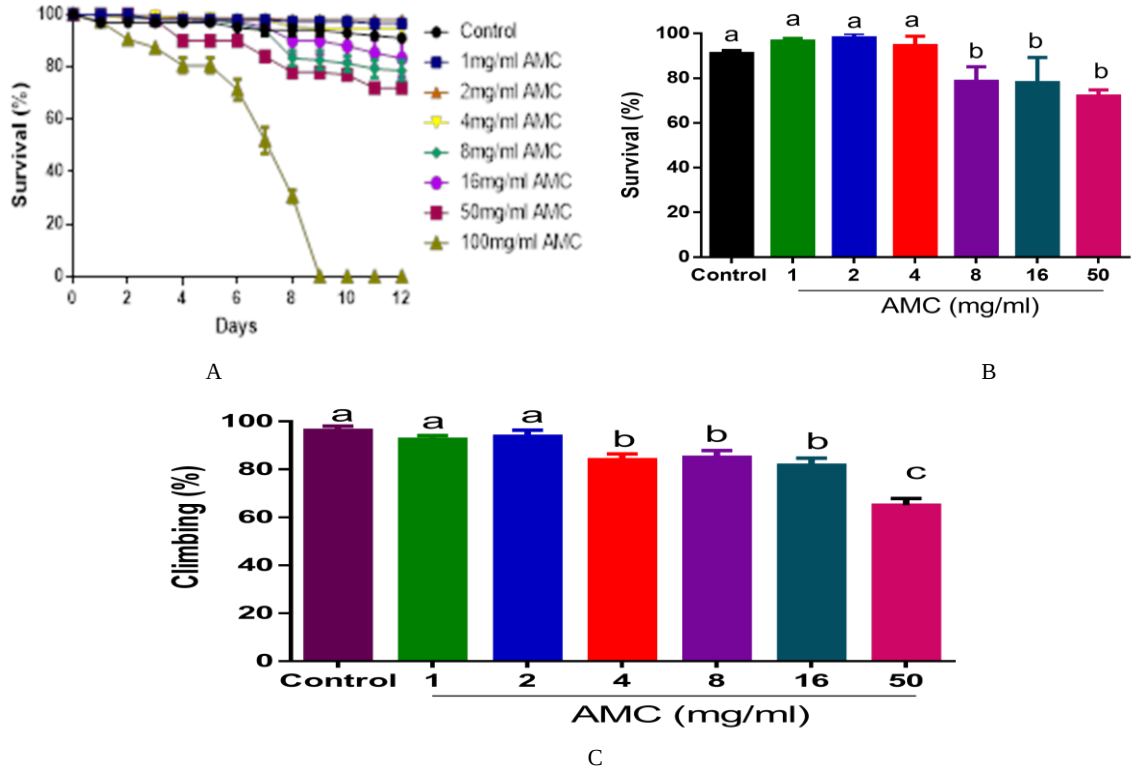

Figure 1: Effect of AMC on survival rate and locomotive behaviour in *D. melanogaster*
The results represent the mean ± SEM of three biological replicates. Bars with different letters are significantly different. ($p < 0.05$). **(A)** survival rate curve; **(B)** survival rate (%) **(C)** Locomotive behaviour after 12 days exposure of *Drosophila melanogaster* to AMC.

Table 1: Effect of AMC on MDA content and antioxidant status

	MDA content (nM)	SOD activity (U/mg protein)	GST activity (U/mg protein)	GSH (µM)
Control	72.61 ± 2.14^a	0.01 ± 0.00^a	0.07 ± 0.01^a	5.44 ± 1.64^a
2 mg/ml AMC	87.61 ± 9.1^a	0.03 ± 0.00^a	0.13 ± 0.01^a	3.69 ± 0.59^a
4 mg/ml AMC	86.54 ± 9.61^a	0.04 ± 0.01^a	0.16 ± 0.02^a	3.11 ± 0.35^a
8 mg/ml AMC	86.54 ± 3.21^a	0.04 ± 0.01^a	0.29 ± 0.07^b	3.17 ± 0.89^a

The results represent the mean ± SEM of three biological replicates. Values on the same columns with different letters are significantly different ($p < 0.05$).

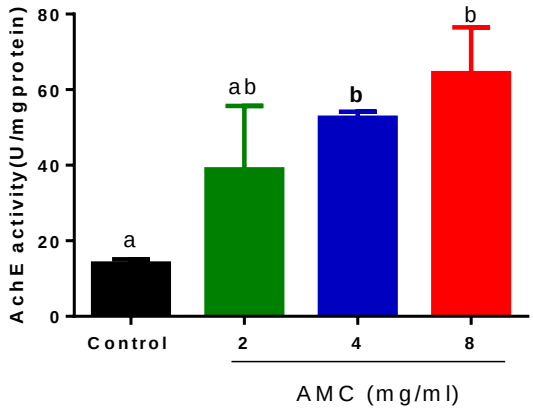

Figure 2: Effect of AMC on acetylcholinesterase activity.
The result represents the mean ± SEM of three biological replicates. Bars with different letters are significantly different ($p < 0.05$).

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.

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