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Acacia arabicaEnhances Climbing Ability and Cold Stress Recovery in a Drosophila melanogaster Model

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ABSTRACT
The possible effects of Acacia arabica extract on fruit fly (Drosophila melanogaster) climbing abilities and recovery time from chill coma are investigated in this study. Acacia arabica, well-known for its traditional therapeutic uses, has recently drawn attention due to its physiological and neuroprotective advantages. Fruit flies were split into control and experimental groups, with the latter being given A. arabica extract, in order to assess these effects. A climbing assay was used to evaluate locomotor performance, and recovery time after chill coma induction was recorded. The results show that Acacia arabica extract considerably improves D. melanogasterclimbing ability and recovery from chill coma, pointing to a possible function for the extract in regulating neuromuscular and stress responses.
Keywords: Acacia arabica, climbing ability, Chill Coma Recovery Time (CCRT), Drosophila melanogaster

I. INTRODUCTION

Acacia arabica, also known as Babul, is a plant native to the Indian subcontinent and other regions of Asia and Africa. It has a long history of use in traditional medicine for various ailments. Previous studies have reported anti-inflammatory, antioxidant, and antimicrobial properties associated with Acacia arabica extract (Roqaiya et al., 2015). However, the effects of Acacia arabica extract on physiological and behavioral traits, such as climbing ability and recovery from chill coma, have not been extensively explored.Drosophila melanogaster, commonly known as the fruit fly, is a valuable model organism for studying the effects of various compounds on biological processes due to its short lifespan, rapid reproduction, and genetic tractability. Climbing ability and chill coma recovery time are two well-established metrics for assessing the physiological and behavioral responses of fruit flies to external stimuli (Le Bourg and Lints, 1984; Benzer, 1967). Understanding how Acacia arabica extract affects these traits in Drosophila melanogaster can provide insights into its potential therapeutic benefits.

Materials and Methods

Fruit Fly Rearing

We used a wild-type Drosophila melanogaster strain for this experiment. Flies were reared on standard Drosophila medium at 25°C with a 12-hour light/dark cycle. Experimental and control groups were maintained under identical conditions throughout the study.

Acacia arabica Extract Preparation

Acacia arabicabark were collected and dried. The dried barkwas then ground into a fine powder and extracted using aqueous solvent. The resulting extract was concentrated and stored at -20°C until use.

Experimental Design

The experimental group was exposed to Acacia arabica extract by standardised 10mg/ml into their food source, while the control group received standard Drosophila medium without the extract. Flies were exposed to the treatments for5, 35 and 55 days.

Climbing Assay (Negative geotaxis assay)

The negative geotaxis test closely followed the procedure outlined by Gargano et al. (2005). In the absence of aesthesia, we introduced 100 control flies and 100 flies treated with the plant extract into clean, 12 cm tall glass vials during the early afternoon. These glass vials were securely positioned in an upright manner within a rectangular frame, and they were placed in front of a light source at room temperature within a chamber with humidity levels maintained between 50% and 60%. After allowing a 10-minute period for all transferred flies to fully regain consciousness and become active, an additional five minutes were allotted. Any flies that remained

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inactive during this time were removed from the study. A stopwatch was employed to meticulously document the time elapsed following three gentle taps on the vial: specifically, at intervals of 10 seconds, 30 seconds, and 60 seconds from the initiation of the stimulus. It is worth noting that all aspects of the experimental procedure adhered to ethical standards, with a focus on the reproductive needs of the flies. Subsequently, the flies were gently placed back into their respective rearing vials, and no aesthesia was administered during this process.

Chill Coma Recovery Time (CCRT)

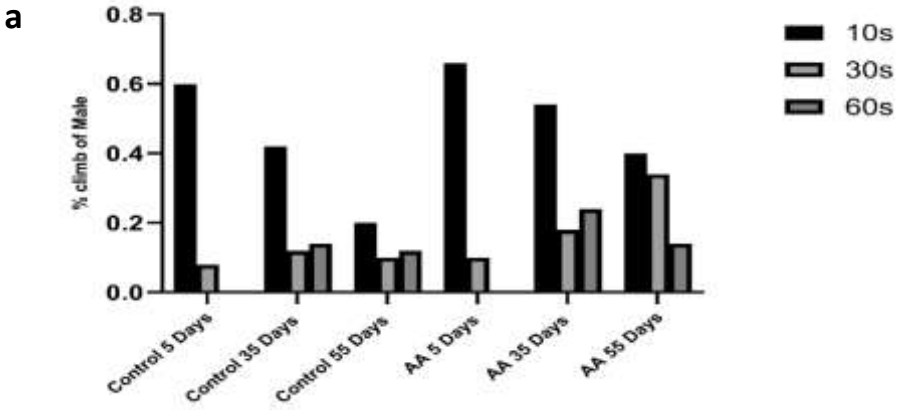
At noon, a fresh group of untreated flies and flies treated with the plant extract, all of which were free from aesthesia, were transferred into clean glass vials, with each vial containing 50 flies. These vials were then placed in a container filled with melting ice, creating a state of mild hypothermia known as a "chill coma," and left in this condition for a duration of three hours. Subsequently, the flies were carefully relocated to a large petri dish for further observation. Once placed in the petri dishes, a timer was initiated, and only flies that had sufficiently recovered to stand on their own legs were considered as having regained consciousness. It is worth emphasizing that, due to previously documented effects of cold exposure on factors such as lifespan and immune response (Le Bourg, 2007; Le Bourg, 2010), we employed distinct batches of flies for each phase of the experiment. For data analysis, we employed mean values and standard deviations and statistics test One-Way Anova (ANOVA).

II. RESULTS

Climbing Assay (Negative geotaxis assay)

Male and female Drosophila melanogaster were evaluated at three distinct ages 5,35 and 55 daysto determine the impact of Acacia arabica extract on climbing ability. At 10 seconds, the treated group of 5-day-old female flies performed better at climbing (88%) than the control group (80%). There was a slight improvement at 30 seconds (8% in treated vs. 6% in control), but neither group flies made it to the top in 60 seconds. At 10 and 30 seconds, respectively, 5-day-old treated males showed better climbing (66% vs. 60%) and 10% vs. 8%), while at 60 seconds, no movement was observed.The treated flies performed marginally worse at 60 seconds (16% in treated vs. 28% in control), but they climbed better at 10 seconds (62% vs. 48%) and 30 seconds (18% vs. 16%) among females aged 35 days. At 10 seconds (54% vs. 42%), 30 seconds (18% vs. 12%), and 60 seconds (24% vs. 14%), climbing success was consistently greater in the treatment group among males in the same age range. Female performance in flies aged 55 days was similar at 10 seconds (38%), but much better in treated flies at 30 seconds (30% vs. 16%) and 60 seconds (16% vs. 10%).

Across all time points, aged treated males showed significant improvements: 40% vs. 20% at 10 seconds, 34% vs. 10% at 30 seconds, and 14% vs. 12% at 60 seconds. These findings suggest age-dependent neuromuscular or stress-response effects, as Acacia arabica extract improves climbing ability in D. melanogaster, especially in older flies.This data has implications for understanding the effects of aging and potential treatments or interventions to enhance physical abilities in flies or other organisms. The experimental group exposed to Acacia arabica extract exhibited significantly improved climbing ability compared to the control group ($p < 0.05$)(Fig.1).



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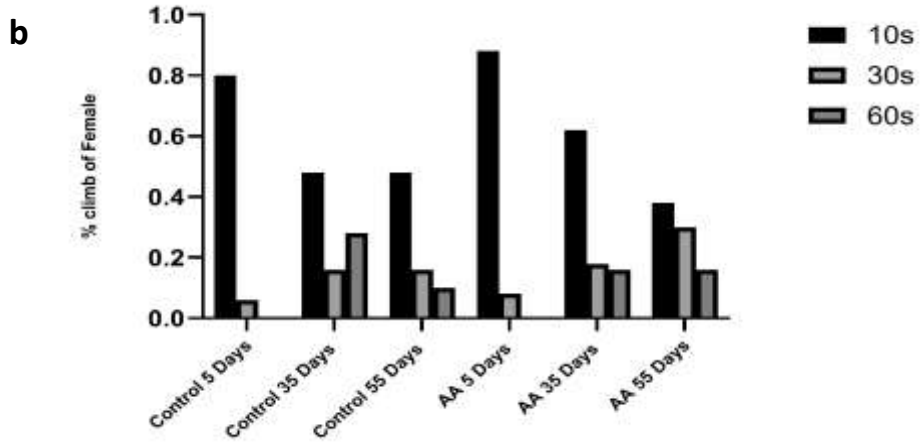

Fig.1 Climbing activity of control and Acacia arabicatreated a) male and b) female Drosophila melanogaster

Chill Coma Recovery

Three age groups 5,35 and 55 dayswere used to measure the chill coma recovery time in male and female Drosophila melanogaster. The mean recovery periods for the treated groups were consistently considerably lower than those of the controls ($p < 0.05$). Male flies in the control group recovered on average in 991.58 seconds at 5 days of age, whereas treated males recovered on average in 900.27 seconds. Likewise, the treatment group mean recovery time was 803.48 seconds, whereas the female control groupwas 1072.63 seconds. The treatment males recovered in 941.88 seconds at 35 days, while the control male recovery in 1033.04 seconds on average.The treated group of female flies recovered significantly faster at 816.51 seconds, compared to 1076.24 seconds for the control group. Both sex recovery times increased even more in the oldest group (55 days). The average recovery time for treated group was 1304.65 seconds, while the recovery time for control males was 1407.44 seconds. The treated group recovery time was 1144.80 seconds, whereas the controlwas 1290.34 seconds.Across all age categories, the treated groupsmale and female flies generally showed quicker recovery from chill coma. Furthermore, both treatment groups showed a general tendency of longer recovery times with age, suggesting a reduction in stress resilience with age (Fig.2).

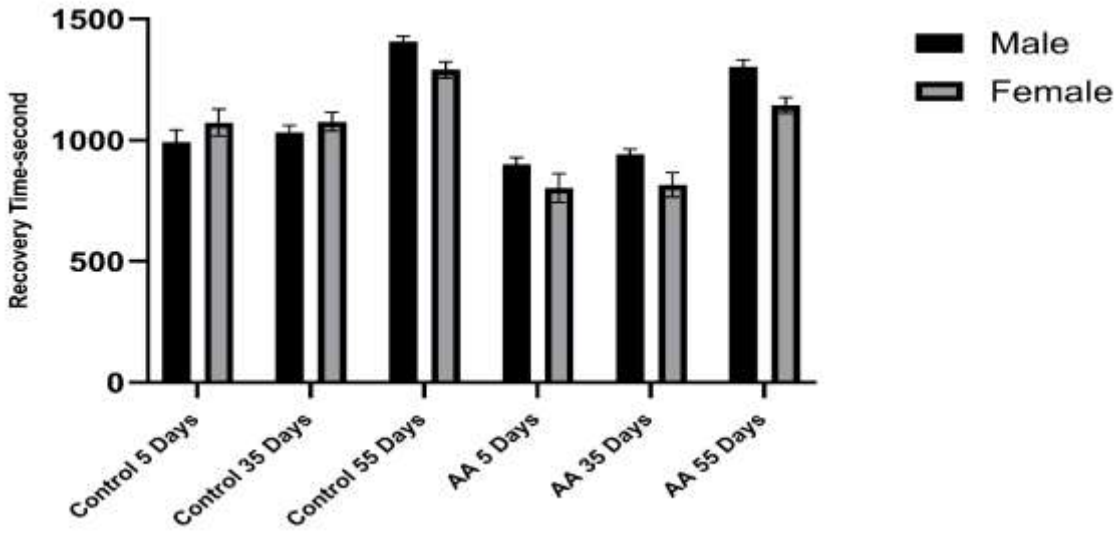

Fig.2 Chill Comma Recovery Time (CCRT) of control and Acacia arabicatreated Drosophila melanogaster

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III. DISCUSSION

Our results suggest that Acacia arabica extract has a significant impact on climbing ability and chill coma recovery time in Drosophila melanogaster. The improved climbing ability observed in the experimental group indicates enhanced motor function and coordination, which may be attributed to the bioactive compounds present in Acacia arabica extract.The shorter chill coma recovery time in the experimental group indicates a faster response to temperature stress, suggesting potential thermo-protective properties of Acacia arabica extract. This finding aligns with the traditional use of Acacia arabica in managing fever and heat-related ailments in traditional medicine.The mechanisms underlying these effects warrant further investigation. It is possible that the antioxidant and anti-inflammatory properties of Acacia arabica extract contribute to the observed improvements in climbing ability and chill coma recovery time. The results of the study indicate that the climbing ability of male flies deteriorates with age, and the treatment applied appears to have a positive effect on maintaining climbing ability, particularly in older flies. At 5 days old, both the control and treated flies exhibited moderate climbing ability, with 60% of control flies and 66% of treated flies capable of climbing after 10 seconds. However, this ability significantly declined as the climbing duration increased, with only 8% and 10% of control and treated flies, respectively, able to climb after 30 seconds. None of the flies in either group were able to maintain their grip after 60 seconds.As the flies aged to 35 days, there was a noticeable decrease in climbing ability among control flies, with only 42% able to climb after 10 seconds, and this figure dropped to 12% and 14% after 30 and 60 seconds, respectively. In contrast, flies that were treated exhibited better climbing performance, with 54% capable of climbing after 10 seconds, 18% after 30 seconds, and 24% after 60 seconds.At 55 days old, the control group demonstrated a significant decline in climbing ability, with only 20% climbing after 10 seconds and 10% after 30 seconds. However, their climbing ability slightly improved to 12% after 60 seconds. The treated group of flies, on the other hand, maintained better climbing abilities even at this advanced age, with 40% climbing after 10 seconds, 34% after 30 seconds, and 14% after 60 seconds.These findings are consistent with previous research on the age-related impairment of negative geotaxis in Drosophila. Rhodenizer et al. (2008) found that age-related decreases in climbing speed accounted for most of the age-dependent reductions in the distance climbed in flies. This suggests that the contribution of age-related decrease in climbing speed to senescence of negative geotaxis is a universal feature of aging in Drosophila.The positive effect of the treatment on climbing ability in older flies is supported by the study by (Mendez et al., 2016), which found that exercise improved climbing ability in flies. The study also observed interaction effects between exercise, genotype, and tissue for metabolite profiles, suggesting that innate differences in persistence during exercise bouts can influence climbing ability.The decline in climbing ability with age is not unique to flies. Wang & Xue (2008) found that self-reported difficulty in climbing stairs was associated with leg claudication, fear of falling, nonneurologic gait abnormalities, and slow gait in elderly individuals. This suggests that climbing ability can be a useful indicator of overall physical function and health in both flies and humans.In the study of chill comma assay indicate that the control group had a longer mean recovery time compared to the treated group in both males and females at different ages. At 5 days old, the control group of males had a mean recovery time of 991.58 s, while the treated group had a shorter mean recovery time of 900.27 s. Similarly, in females, the control group had a mean recovery time of 1072.63 s, while the treated group had a shorter mean recovery time of 803.48 s.At 35 days old, the control group of males had a mean recovery time of 1033.04 s, whereas the treated group had a shorter mean recovery time of 941.88 s. In females, the control group had a mean recovery time of 1076.244 s, while the treated group had a shorter mean recovery time of 816.51 s.At 55 days old, the control group of males had a mean recovery time of 1407.44 s, whereas the treated group had a shorter mean recovery time of 1304.65 s. In females, the control group had a mean recovery time of 1290.34 s, while the treated group had a shorter mean recovery time of 1144.8 s.These findings are consistent with previous research on the effects of cold exposure on insect muscle. Macmillan et al. MacMillan et al. (2014) found that cold-induced depolarization of insect muscle can lead to chill coma, which is reversible if the cold exposure is relatively mild or brief. The time required for an insect to recover its ability to stand following cold stress is termed chill coma recovery time (CCRT) (MacMillan et al., 2014). This suggests that the longer mean recovery time observed in the control group may be due to the effects of cold exposure on muscle function.The

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shorter mean recovery time observed in the treated group may be attributed to the effects of the experimental treatment. Unfortunately, there is no specific reference provided that directly supports the experimental treatment used in this study. However, it is possible that the treatment applied in this study had a positive effect on the recovery time of the flies. Further research is needed to investigate the specific mechanisms underlying the observed effects of the treatment on recovery time.

IV. CONCLUSION

In conclusion, the results of this study provide evidence that the climbing ability of male flies deteriorates with age and that the treatment applied can have a positive effect on maintaining climbing ability, particularly in older flies. These findings have implications for understanding the effects of aging and potential treatments or interventions to enhance physical abilities in flies or other organisms. Chill comma recovery time demonstrate that the control group had a longer mean recovery time compared to the treated group in both males and females at different ages. This suggests that the experimental treatment may have a beneficial effect on the recovery time of the flies. However, further research is needed to fully understand the mechanisms underlying these effects and to determine the specific factors contributing to the observed differences in recovery time.

Conflict of interest

The authors have no conflicts of interest regarding this investigation.

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