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Original Paper

Curcumin and nanocurcumin effects on weight, negative geotaxis, total protein, protein profiling by SDS-PAGE, and lifespan in *Drosophila melanogaster*

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ABSTRACT

Curcumin, a polyphenol extracted from turmeric rhizomes, has shown promising effects in several biological systems, including model organisms. This study investigated the effects of curcumin and nanocurcumin on weight, negative geotaxis, total protein, protein profiling via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and lifespan in *Drosophila melanogaster* as a model organism. Curcumin and nanocurcumin were individually added to the *Drosophila melanogaster* medium at concentrations of 0.5, 1, 5, and 10 mg/g of media, alongside a control group, with three replicates for each concentration. ANOVA and Tukey's post hoc test were performed for statistical analysis. The study revealed that curcumin and nanocurcumin had statistically significant effects on multiple parameters, with nanocurcumin showing a greater impact than curcumin and the control group, suggesting that the nano form has higher biological effectiveness and better cellular interaction. As evidenced by an extended *Drosophila melanogaster* lifespan ($P < 0.001$) and increased weight ($P < 0.01$), negative geotaxis ($P < 0.01$), and total protein ($P < 0.002$), an increase in negative geotaxis indicates improved neuromuscular coordination, while increases in weight, total protein, and lifespan may reflect better metabolic activity or nutrient absorption. In addition, curcumin and nanocurcumin significantly altered the SDS-PAGE protein profile, suggesting a potential impact on protein expression that may be linked to gene regulation. These findings demonstrate that curcumin and nanocurcumin have a pronounced impact on physiological and biochemical parameters, with nanocurcumin exerting a stronger effect, making them promising options for biological applications.

Keywords: *Curcumin, Drosophila melanogaster, Nanocurcumin., Negative geotaxis, Weight.*

1 INTRODUCTION

Curcumin is a natural biopolymer extracted from the rhizomes of the Curcuma longa plant. It is responsible for turmeric's yellow color and is considered its active ingredient [1, 2]. Curcumin is characterized by unique properties, such as antioxidant and antimicrobial activities, improved neurological function, and protection of the heart and blood vessels, making it an important substance for therapeutic, environmental, and other applications [3]. However, one of the most important challenges limiting its applications is poor intestinal absorption due to its low water solubility [4].

Nanocurcumin is the nanoscale form of curcumin, produced in several ways to yield nanometer-sized particles that can improve its properties, increase bioavailability, and enhance its biological effects [5]. Curcumin and nanocurcumin have gained widespread attention in recent years due to their potential roles in enhancing physiological and metabolic functions [3].

Aging is defined as a decline in performance and fitness with progressing age. The fruit fly (*Drosophila melanogaster*) is an ideal model organism for scientific research due to its ease of breeding, short life cycle, and genetic similarity to higher organisms. It has been widely used in nutritional studies and biotoxicity research, making it suitable for evaluating the effects of curcumin and nanocurcumin on various physiological processes. Aging affects multicellular organisms and is linked to diseases such as diabetes, cancer, and heart disease. To combat aging, plant-derived compounds such as curcumin may slow age-related deterioration [6, 7].

Previous studies have investigated the effects of curcumin on fruit fly weight and negative geotaxis in the model organism *Drosophila melanogaster* [8–10]. In addition, a previous study evaluated curcumin in fruit flies using concentrations of 0.5 and 1.0 mg/g of media [11]. These studies indicated that curcumin influences physiological aspects of the organism; however, they did not address the effects of the nano form of curcumin or a concentration gradient. In contrast, the present study aims to evaluate the effects of both curcumin and nanocurcumin on selected physiological parameters in fruit flies using concentration gradients, thereby enabling a deeper understanding of the responses.

This research aims to evaluate the effects of curcumin and nanocurcumin on weight and negative geotaxis in *Drosophila melanogaster*.

2 MATERIALS AND METHODS

2.1 Preparation of nanocurcumin for analysis and characterization

The preparation of nanocurcumin (provided by Nanochemazone, Canada) and its physical characterization, including scanning electron microscope (SEM), X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDX), UV–visible spectroscopy (UV-vis), and chromaticity analyses, were conducted according to methods detailed in a previous study [12].

2.2 *Drosophila melanogaster* collecting, stock, and medium preparation

Drosophila melanogaster insects were collected using traps containing fermented fruits (bananas, apples, and oranges) in Anbar Governorate. The trap was left in the garden for four consecutive days and then transferred to the laboratory after its opening was covered with gauze to prevent flies from escaping, as shown in Figure 1. The collected insects were then sent to the Natural History Museum of the University of Baghdad for diagnosis. The culture medium was prepared according to the method described in [8] with some modifications. The medium was prepared by dissolving 3.6 g of active dry yeast, 3 g of agar, 16 g of corn flour, and 13 g of sucrose in 200 mL of distilled water, then mixing with a magnetic stirrer. It was then sterilized in an autoclave. After it became lukewarm, 900 μL of propionic acid was added, mixed well, and left to solidify. *Drosophila melanogaster* was transferred to the medium and incubated at 25 ± 2 °C to produce a new generation.

Fig. 1 *Drosophila melanogaster* trap

2.3 Evaluation of the effect of curcumin and nanocurcumin on the weight of *Drosophila melanogaster*

After obtaining the new generation of fruit flies, nutritional media were prepared using the same ingredients described above, with curcumin added. Curcumin (molecular weight 368.38) was supplied by Avonchem, UK, and applied at concentrations of 0.5, 1, 5, and 10 mg/g of medium after dissolving in 20% ethanol to ensure it mixed well with the medium. Three replicates were used for each concentration. Another group contained nanocurcumin at the same concentrations. Control groups were prepared similarly without adding curcumin or nanocurcumin. After the media solidified, five males and five females aged 3 days were transferred to each replicate. After feeding with curcumin and nanocurcumin for 10 days, the flies were anesthetized with diethyl ether, weighed on a sensitive balance, and the weights were recorded for statistical analysis. The experiment was repeated to verify the accuracy of the results. Media were prepared as described in [13].

2.4 Evaluation of the effect of curcumin and nanocurcumin on the negative geotaxis of *Drosophila melanogaster*

The test was conducted after the flies were prepared. Ten flies (5 males and 5 females), aged 3 days, were moved and fed with a medium that was prepared similarly, as mentioned above. Curcumin and nanocurcumin were added separately at the same concentrations (0.5, 1, 5, and 10 mg/g of medium), with a control group, and the flies were maintained for 10 days. Diethyl ether was then used to anesthetize the flies, which were transferred to a graduated cylinder, set to a height of 6 cm, and the cylinder nozzle was closed. After the flies recovered from anesthesia (10 minutes), a light tap was made on the cylinder surface to cause the flies to fall to the bottom; the timer was started, and the number of flies that crossed a height of 6 cm within 8 seconds was recorded. The process was repeated three times at 3-minute intervals. The method described in [14] was followed with some modifications.

2.5 Evaluation of the effect of curcumin and nanocurcumin on total protein of *Drosophila melanogaster* by the Bradford method

The 3-day-old flies were fed on a nutritional medium containing curcumin at concentrations of 0.5, 1, 5, and 10 mg/g of medium and another nutritional medium containing nanocurcumin at the same concentrations. With the presence of a control group and three replicates for each concentration, the feeding period continued for 10 days, after which the flies were collected and crushed using a homogenizer for analysis. The protein concentration of the sample was determined using a commercial kit (Bioquochem, catalog No. KB03003) according to the manufacturer's instructions.

2.6 Evaluation of the effect of curcumin and nanocurcumin on protein profile patterns using SDS-PAGE

The same flies' extracts were prepared for total protein analysis by the Bradford method, as described above, and were used for SDS-PAGE analysis. Proteins were separated using a commercial kit (SolarBio, catalog No. p 1200) according to the manufacturer's instructions.

2.7 Evaluation the effect of curcumin and nanocurcumin on lifespan of *Drosophila melanogaster*

The method described in [14] was followed with some modifications. After obtaining the new generation of fruit flies, nutritional media were prepared as described previously, with the addition of curcumin at different concentrations (0.5, 1, 5, and 10 mg/g of medium after dissolving it in 20% ethanol to ensure it was well mixed with the medium), with three replicates for each concentration, and another group containing nanocurcumin at the same concentrations. A control group also contained 20% ethanol without adding curcumin or nanocurcumin. Ten flies (5 males and 5 females), 3 days old, were transferred to each replicate, ensuring that temperature and humidity were controlled and the nutritional medium was changed every five days. Deaths were recorded every day until the last fly died to monitor the effect of curcumin and nanocurcumin on lifespan. The experiment was repeated to ensure the accuracy of the results.

2.8 Statistical analysis

All data were analyzed using SPSS version 23. The mean and standard deviation were included in the results. Tukey's test and a one-way ANOVA were used to compare the groups. A significant P value was defined as less than 0.05.

3 RESULTS AND DISCUSSION

3.1 Impact of curcumin and nanocurcumin on weight

Both curcumin and nanocurcumin exhibited an influence on the weight of *Drosophila melanogaster*, as presented in Figure 2, compared with the control group. An increase in weight was observed with curcumin and nanocurcumin treatment, with a greater effect of nanocurcumin; however, the increase began to decline at higher concentrations (starting at 5 mg/g of media) ($F = 3.691$, $P < 0.01$).

The results indicated that the insect's weight increased with increasing concentrations of both curcumin and nanocurcumin up to a certain level (5 mg/g of medium), then decreased at higher concentrations. This indicates a dual effect of the substance, depending on concentration: it improved overall insect growth at low and medium concentrations, which may be due to its antioxidant properties, thereby reducing cellular oxidative stress and improving physiological functions associated with growth. In contrast, at high concentrations, the decrease may be due to metabolic stress resulting from the accumulation of the substance within tissues, which can, in turn, disrupt vital processes. From Figure 2, we observe that nanocurcumin is more effective than the control group and raw curcumin, reflecting its higher absorption and biological activity due to its smaller molecular size. The weight gain resulting from treatment with curcumin and nanocurcumin suggests that it may be due to enhanced protein synthesis in the insect body. This may be related to the role of curcumin and its derivatives in improving metabolic efficiency and activating protein synthesis pathways, in addition to reducing oxidative stress, which provides a suitable internal environment for growth [15, 16].

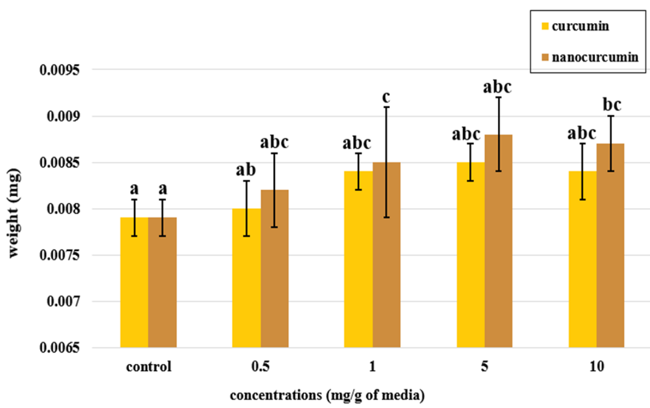

Fig. 2 Following ten days of exposure to curcumin and nanocurcumin, using 4 different concentrations alongside a control group (3 replicates for each one). The treatment shows impact on weight. Different letters above bars identified by Tukey's test (P<0.05) show the significant difference.

3.2 Impact of curcumin and nanocurcumin on negative geotaxis

As shown in Figure 3, curcumin and nanocurcumin affected negative geotaxis compared with the control group. An increase in the climbing ability of *Drosophila melanogaster* was observed following treatment with curcumin and nanocurcumin, with a greater effect of nanocurcumin ($F = 3.706$, $P < 0.01$). The effect then stabilized at higher concentrations.

Negative geotaxis in *Drosophila* refers to their natural behavior of climbing upward against gravity after being tapped down to the bottom of a container. This upward climbing is an innate escape response and is used as a measure of locomotor ability and aging in fruit flies. The strength of this climbing behavior tends to decline with age. The test results shown in Figure 3 indicate that curcumin and nanocurcumin increased the motor activity of the insect compared with the control group, with a greater effect for nanocurcumin. This may be due to the small size of its molecules, which contributes to increased efficiency in penetrating cell membranes and increasing solubility, thus facilitating access to muscle and nervous tissues and improving their function, as curcumin is characterized by compounds that contribute to reducing oxidative stress. Nanocurcumin can also contribute to improved movement by enhancing nerve transmission through its ability to improve nerve signaling. It also raises protein levels, which contribute to strengthening muscle fibers and increasing the efficiency of contraction necessary for climbing, thus protecting nerves and muscles. The stabilization of the effect at high concentrations may indicate saturation of the biological effect or the onset of adverse effects. This is consistent with many previous studies [17–19].

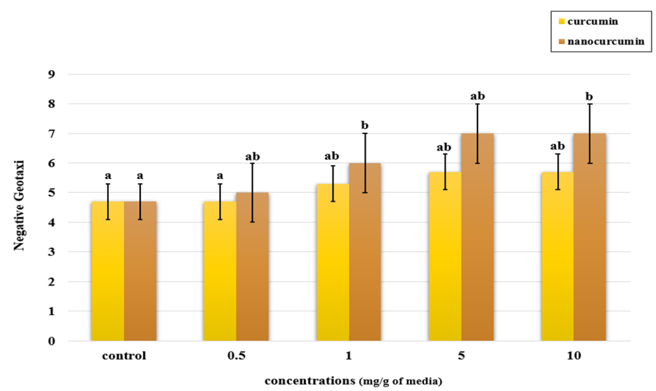

Fig. 3 Following ten days of exposure to curcumin and nanocurcumin, using 4 different concentrations alongside a control group (3 replicates for each one). The treatment shows an impact on negative geotaxis. Different letters above bars identified by Tukey's test (P<0.05) show the significant difference.

3.3 Impact of curcumin and nanocurcumin on total protein

As shown in Figure 4, treatment of *Drosophila melanogaster* with curcumin and nanocurcumin was statistically significant by ANOVA ($F = 5.362$, $P < 0.002$). The effect was clear at 0.5 mg/g of media for nanocurcumin, whereas 1 mg/g of media showed the clearest effect for curcumin.

The results of total protein analysis, shown in Figure 4, are consistent with previous studies [20, 21]. Curcumin and nanocurcumin treatments significantly increased total protein levels in insects, which may be due to their antioxidant properties, which reduce ROS levels and protect cellular proteins from oxidation and damage [22]. Curcumin may also stimulate protein biosynthesis pathways by improving mitochondrial activity and increasing energy production [23]. The greater effectiveness of nanocurcumin may be due to its improved physical properties, which increase tissue permeability and enhance its ability to stimulate biological responses. Overdose can decrease total protein concentration and disrupt cellular balance, making the benefits of curcumin and nanocurcumin dose-dependent.

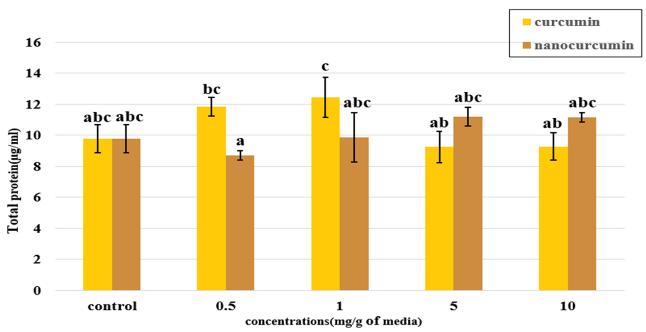

Fig. 4 Following ten days of exposure to curcumin and nanocurcumin, using 4 different concentrations alongside a control group (3 replicates for each one). The treatment shows impact on total protein. Different letters above bars identified by Tukey's test (P<0.05) show the significant difference.

3.4 Impact of curcumin and nanocurcumin on protein profile using SDS PAGE

Figure 5 shows that curcumin and nanocurcumin significantly altered protein band patterns in the treated groups, with new bands appearing or disappearing and band thickness increasing or decreasing. Band B was present in both the control and curcumin-treated groups; however, it disappeared in the nanocurcumin-treated group. Band C was present in both treated groups and the control group, but disappeared in the curcumin-treated group at a concentration of 0.5 mg/g. In the nanocurcumin-treated group, band C showed a reduction in thickness, followed by an increase at a nanocurcumin concentration of 10 mg/g of media.

The results show that treatment with curcumin and nanocurcumin significantly modified the protein patterns of *Drosophila melanogaster*. The emergence of new bands may indicate activation of the expression of new proteins associated with adaptive response pathways, such as the antioxidant enzymes (SOD) and (CAT), indicating stimulation of cellular protection pathways against oxidative stress. The disappearance of some bands may be due to the inhibition of protein expression or to a decline in their production, resulting from the stimulation of protein degradation mechanisms. The increase in the thickness of some bands may be linked to enhanced expression of functional proteins. In contrast, a decrease in the thickness of some bands may reflect selective inhibition of proteins associated with normal metabolic pathways. It is worth noting that the effect of nanocurcumin was more prominent due to its high permeability through cell membranes, which gives it greater opportunity to cause change. This agrees with findings reported in previous studies [24–26].

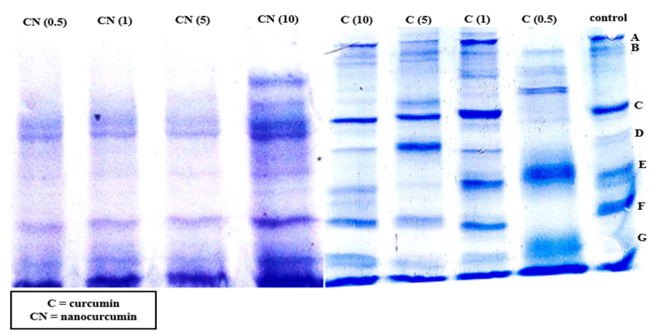

Fig. 5 Effect of curcumin and nanocurcumin on protein profile in *Drosophila melanogaster* via SDS-PAGE

3.5 Impact of curcumin and nanocurcumin on the lifespan of *Drosophila melanogaster*

Figure 6 indicates the impact of curcumin and nanocurcumin on fruit fly lifespan. It is clear that nanocurcumin had a greater effect than curcumin and the control group, and that the greatest treatment effect was observed at low and medium concentrations. After that, the effect reversed and lifespan began to decrease at high concentrations, with a greater decrease for nanocurcumin than for raw curcumin ($F = 52.545$, $P < 0.001$).

Our results indicated that curcumin and nanocurcumin have a biphasic effect on fruit fly lifespan, where lifespan gradually increases up to a certain concentration and then begins to decrease at higher concentrations. This is a common occurrence for compounds with antioxidant properties: they elicit a beneficial response at low and medium concentrations but can cause stress at high doses. The positive effect of treatment may be due to its antioxidant activity, which enhances cellular resistance to oxidative stress, one of the most important causes of aging in Drosophila. However, exceeding the optimal concentration can cause adverse effects, perhaps due to the compound's accumulation. The greater effectiveness of nanocurcumin compared with curcumin may be due to its physicochemical properties, which enhance its biological activity. This is consistent with a previous study [27–29].

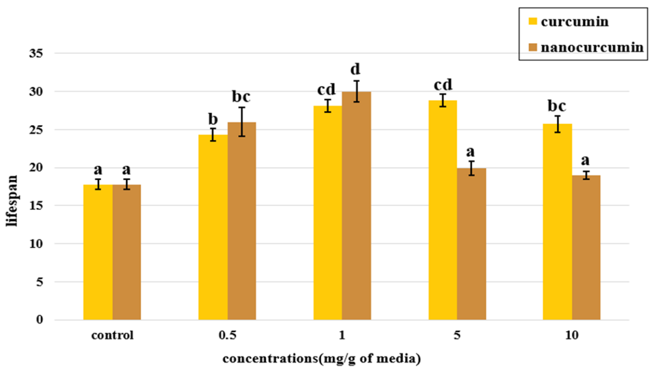

Fig. 6 Following ten days of exposure to curcumin and nanocurcumin, using 4 different concentrations alongside a control group (3 replicates for each one). The treatment shows an impact on the lifespan. Different letters above bars identified by Tukey's test ($P<0.05$) show the significant difference.

4 CONCLUSION

This study demonstrated that curcumin and nanocurcumin had significant effects on lifespan, weight, negative geotaxis, total protein, and protein profile in *Drosophila melanogaster*, with a stronger effect for nanocurcumin than for curcumin or the control group. Nanocurcumin effectively extended lifespan, increased weight and total protein, and improved negative geotaxis with statistically significant results. In addition, SDS-PAGE analysis showed noticeable changes in the protein profile, especially with nanocurcumin treatment. The effectiveness of the materials (curcumin and nanocurcumin) is dose-dependent for optimal effects.

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Data availability

N/A

DECLARATIONS

Conflict of interest

I affirm that no conflict of interest exists.

Consent to publish

N/A

Ethical approval

N/A

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