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RESEARCH ARTICLE

Investigating the Combinatorial Effects of Nanoparticles and Antioxidant Compounds on *Drosophila Melanogaster*

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ABSTRACT: Nanoparticles have been reported to potentially cause a detrimental effect on fertility and physical fitness in humans. This is a concern due to an increasing exposure of nanoparticles in our daily environment. Previous studies have also shown that antioxidant compounds increased longevity and fertility in model organisms. In this study, we used a *Drosophila melanogaster* model to examine the combinatorial effects of nanoparticles and antioxidants. First, using a climbing motility assay and a larvae count fertility assay, we looked at three commonly found nanoparticles: gold, silver, and titanium, and established a working concentration where they exerted a negative influence on the physical fitness and fertility of *Drosophila* adult flies. We then determined the beneficial effect of antioxidants melatonin, N-acetylcysteine (NAC), and quercetin. Subsequently, we examined the combined effects of nanoparticles and antioxidants on *Drosophila* physical fitness and fertility. Furthermore, we examined the transgenerational effect of these chemical combinations on the first generation of progeny flies. In our studies, we found that, to varying degrees, each nanoparticle and antioxidant has a detrimental or beneficial effect, respectively, on both mobility and fertility of *Drosophila melanogaster* transgenerationally, with silver nanoparticles being the most detrimental, and melatonin being the most beneficial.

KEYWORDS: Biochemistry, Medical Biochemistry, Nanoparticles, Antioxidants, *Drosophila melanogaster*.

Introduction

Nanoparticles (NPs) have biological impacts that raise sizable concerns due to their increasing prevalence in industrial, medical, and consumer applications. While several nanoparticles are biocompatible and have revolutionized medical and industrial fields, metal-based nanoparticles have displayed potentially hazardous effects on biological systems. These particles, as a result of their minuscule size, have the potential to disrupt crucial physiological processes via direct interaction with cellular structures.[1-3] Research has shown that metal-based nanoparticles, such as gold (Au), silver (Ag), and titanium dioxide (TiO2), can exert detrimental effects on the fertility, development, and overall health of organisms such as *Drosophila melanogaster*. [4-7]

Drosophila melanogaster was the ideal model organism for this project for various reasons. Its short life cycle, fecundity, and genomic structure have made it desirable for a large portion of toxicological studies.[8] However, its most attractive trait for this project was its commercial availability to the general public. *Drosophila melanogaster* is useful for observing the toxicological impacts of NPs and has demonstrated severe developmental delays in response to exposure to TiO2 NPs.[9,10] Previous research has also shown that larval stages of *Drosophila melanogaster* are particularly susceptible to nanoparticle-induced genotoxicity.[11] This genotoxicity can result in aberrant phenotypes, as well as increased susceptibility to DNA damage and behavioral changes that could alter reproductive efficacy over generations. These findings are concerning, especially concerning the safety considerations of these materials in consumer products.

Numerous antioxidants have been the subject of the limelight in recent years. Commercially available compounds such as N-acetylcysteine (NAC), melatonin, and quercetin show promise in oxidative stress mitigation.[12, 13] NAC acts as a glutathione precursor and is applicable in detoxification.[14,15] Melatonin and quercetin, in addition to their antioxidant properties, have been found to ameliorate the neurotoxic effect induced by TiO2 NPs exposure, potentially allowing them to have a similar impact on the neurotoxic effect of AgNPs.[16-19]

This study aims to evaluate and compare the effects of Au, Ag, and TiO2 nanoparticles on the physical fitness and fertility of *Drosophila melanogaster*, while simultaneously measuring the extent to which antioxidants, NAC, melatonin, and quercetin, can mitigate the adverse effects. We hypothesize that Beneficial compounds such as NAC, quercetin, and melatonin can reverse the detrimental effects from nanoparticles such as silver, gold, and titanium dioxide, on fertility and physical capabilities of *Drosophila melanogaster*. Using established mobility and fertility assays, the study aims to contribute to a deeper understanding of nanoparticle toxicity and possible counteractants.

Methods

Drosophila melanogaster maintenance:

Wingless Drosophila melanogaster specimens were obtained from a commercial supplier, Josh's Frogs, and maintained under strictly controlled environmental conditions (Figure 1). The fruit flies are maintained on a culture medium that was obtained from the same manufacturer. Josh's Frogs Culture media is primarily powdered-potato-based, while using vitamins and antifungal ingredients. The powdered culture media

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were reconstituted with water according to the manufacturer's instructions. The flies were housed at a steady temperature of 25°C with a photoperiod consisting of 16 hours of light and 8 hours of dark. A regulated thermal environment was established inside a 20-gallon glass reptile terrarium equipped with a standard heating apparatus. Humidity of the thermal environment was maintained at 65%. Temperature and humidity were monitored using a digital thermometer and a smart hygrometer used in conjunction.

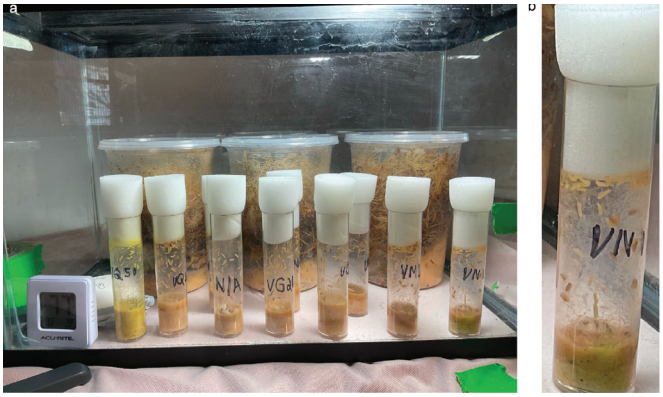

Figure 1: Images of the experimental set up.(a) A controlled tank environment at 25 deg Celsius containing culture vials labelled by food conditions. (b) A culture vial containing food culture and *Drosophila melanogaster*.

Reconstitution of chemicals:

To assess the impact of the various chemicals, melatonin (Sierra Life Sciences), N-Acetyl L-Cysteine (NAC) (Bulk-Supplements.com), quercetin dihydrate (Micro Ingredients), gold (Au) nanoparticles (NutriNoche), silver (Ag) nanoparticles (XFNANO), and titanium dioxide (TiO2) nanoparticles (Alexes) were obtained from reputable vendors in powdered or liquid form. From a literature search, three different concentrations were tested for each chemical.[9,21-25] The chemicals are mixed thoroughly into the culture media reconstituted as described above. Three replicates were performed for each chemical condition using identical 95mm polystyrene culture vials. Chemicals were dissolved in water as a stock solution. Serial dilutions were then used to create solutions of applicable concentrations.

Selection of optimal chemical concentrations:

The prepared solutions were thoroughly incorporated into the culture media within the designated culture vials, ensuring uniform exposure for all experimental groups. Experimental groups were composed of ten flies per culture vial, with a 5:5 male-to-female ratio. The flies were placed into 95mm polystyrene vials containing a culture medium supplemented with precise concentrations of experimental compounds. These vials were labeled according to their experimental conditions and maintained within the controlled environment. Following the initial exposure period, the optimal concentration was determined for each chemical based on maximum observable effects on the flies while minimizing lethality (Table 1). Only the optimal conditions (highest concentration with no observed fly fatalities) of each chemical were used for further studies. Vials that contained suboptimal concentrations were not analyzed further. This process ensured that all experiments utilized the most effective dosages for measurement (Table 2)

Table 1: List of chemicals used and the concentrations tested. The highest concentration with no fatalities observed were used in subsequent experiments.

Chemical used	Concentration	Fatalities observed	Best?
NONE (Food only)	NONE (Food only)	No	**Yes**
NAC	0.1 mg/mL	No	**Yes**
NAC	1 mg/mL	Yes	No
NAC	5 mg/mL	Yes	No
Quercetin	0.1 mg/mL	No	**Yes**
Quercetin	0.5 mg/mL	Yes	No
Quercetin	5 mg/mL	Yes	No
Melatonin	0.01 mg/mL	No	No
Melatonin	0.1 mg/mL	No	**Yes**
Melatonin	0.5 mg/mL	Yes	No
Titanium	0.0014 mg/mL	No	**Yes**
Titanium	0.014 mg/mL	Yes	No
Titanium	0.14 mg/mL	Yes	No
Gold	1 ppm	No	No
Gold	20.5 ppm	No	**Yes**
Gold	30 ppm	Yes	No
Silver	0.1 mg/mL	No	**Yes**
Silver	1 mg/mL	No	No
Silver	10 mg/mL	Yes	No

Table 2: Combinations of Antioxcidants and nanoparticles used.

Condition	Reversal Compound	Nanoparticle
ALPHA	FOOD ONLY	FOOD ONLY
A	NAC	Titanium
B	NAC	Gold
C	NAC	Silver
D	Quercetin	Titanium
E	Quercetin	Gold
F	Quercetin	Silver
G	Melatonin	Titanium
H	Melatonin	Gold
I	Melatonin	Silver

Experimental Groups:

There are four main experimental groups used in this experiment, each of which is staggered apart by a week (Figure 2). P1 refers to the first experimental group for the parent generation and contains all of the individual culture vials that test how each chemical alone affects *Drosophila melanogaster* (Figure 2, Table 1). Three replicates were performed for each chemical condition. P2 refers to the second experimental group of the parent generation, which is subjected to a different combination of chemicals (Figure 2, Table 2). The combined conditions examine whether each nanoparticle's effect on *Drosophila melanogaster* can be mitigated by each antioxidant reversal compound. Due to the large number of permutations, only two replicates of each vial are used in P2. F1 refers to the first experimental group of the filial generation. F1 consists of the offspring of P1 that matured in an environment with only food and no added chemicals. The goal is to measure the potential transgenerational effects that each chemical has on the progeny after the parents were exposed to chemicals. The final group is F2, the second group in the filial generation. F2

is the offspring of P2 that matured in a food-only medium with no chemicals. Thus, this condition examines whether the transgenerational effect of each nanoparticle on *Drosophila melanogaster* can be mitigated by the antioxidant reversal compounds. All experimental groups contain at least one control vial that only contains regular food. The conditions with different combinations of chemicals are labeled A-I in Table 2.

Physical mobility assay:

The locomotor activity of the flies was assessed daily for five days. Five randomly selected files from each condition were placed into a fresh empty vial, where they were allowed a brief acclimatization period of 5 minutes. Following acclimatization, the vial was gently tapped so that the flies were at the bottom of the vial. The number of flies that successfully climbed to three-quarters of the vial's height within 1 minute was recorded. This procedure is repeated for replicates of each condition daily to monitor the changes over 5 days.

Fertility Assay:

Fertility was assessed after the physical mobility assay. On the sixth day, all adult flies are removed from each experimental group, and the larval progeny are counted to assess reproductive output. For the parent generation, the adults are placed into clean culture vials containing normal culture medium and given 4 days for reproduction before being removed and disposed of. The new larvae in the food-only vials are given 8 days to develop into adults before being transferred, in groups of ten with a 5:5 male-to-female ratio, into new culture vials. These new cultures are the first filial generation. They are then assessed under the same 6-day timeline as the previous generation to assess fertility. The remaining adult and larva vials are disposed of. All culture vials containing flies, after their usage, were frozen overnight, wrapped in disposable paper towels, and discarded via standard disposal channels.

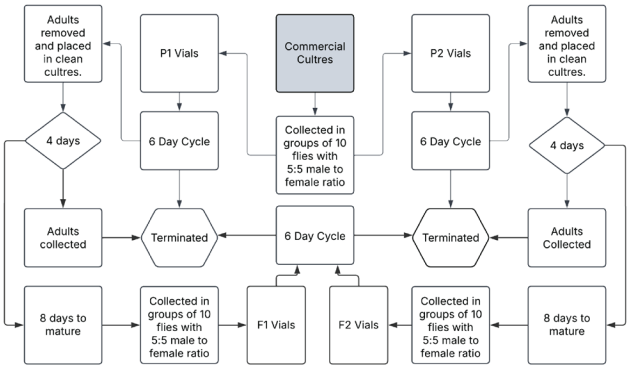

Figure 2: Flowchart illustrating experimental procedures and naming of different groups of files.

Results and Discussion

Optimization of Chemical Concentrations to be used:

The optimal concentrations of each chemical used in the experiment were determined based on the largest impact without fatalities. Table 1 shows all chemical concentrations tested in the initial exposure. Even for the beneficial antioxidant compounds melatonin, NAC, and quercetin, all chemicals resulted in fatalities when administered in doses of high concentrations. This initial exposure was done using the same flies that would later be assayed. Suboptimal concentrations and their duplicates were subsequently disposed of prior to the start of the experiment. The optimal chemical concentrations utilized for the subsequent experiments are as follows: melatonin 0.1mg/mL, N-Acetyl L-Cysteine (NAC) 0.1mg/mL, quercetin dihydrate 0.1mg/mL, gold (Au) nanoparticles 20.5ppm (or 0.025mg/mL), silver (Ag) nanoparticles 1mg/mL, and titanium dioxide (TiO2) nanoparticles 0.00014mg/mL (Table 1).

Group P1 – Mobility Assay:

In the control group of the first parent generation P1, 4 out of 5 flies climbed to ¾ of the tube in the mobility assay, which is in line with the standard geotaxis of *Drosophila melanogaster*. Flies kept in food containing antioxidants showed a higher proportion of flies climbing than the control group, with mel-atonin being the highest by far in the initial days, followed by NAC and quercetin. Flies in all 3 antioxidant groups showed better mobility performance than the control group.

For the nanoparticles, flies grown in AuNPs conditions performed the worst out of all conditions, with as low as an average of 2 displaying geotaxis during the later days of their testing cycle. TiO2 had similar results to AuNPs; however, it did not decline as rapidly in its later days. Silver had the least effect on *Drosophila melanogaster* as files in the AgNPS showed the highest mobility out of all 3 NPS conditions, but still low-er than the control group (Figure 3). This was surprising as silver's neurotoxic effect was reported to have a significant det-rimental effect on the

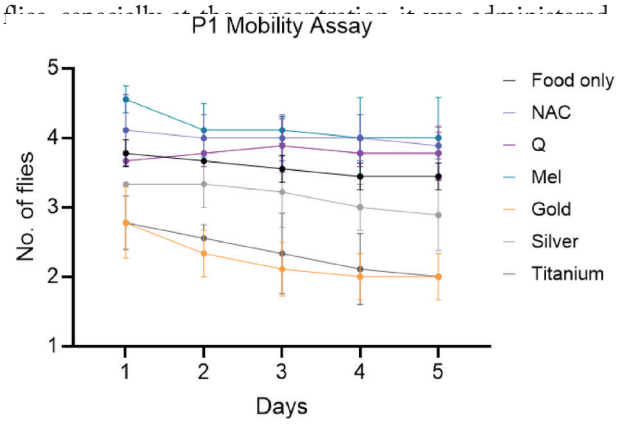

Figure 3: Mobility assy of parent flies exposed to individual compounds. Files exposed to nanoparticles exhibit significant decreased mobility from control (food only) except silver. For antioxidants, no significant changes were observed except in the condition with melatonin.

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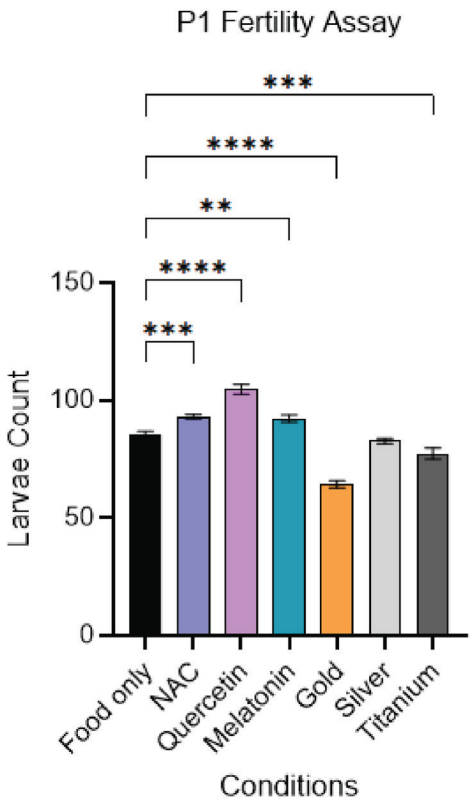

Figure 4: Fertility assay counting larvae from parent flies exposed to individual compounds. Flies exposed to nanoparticles exhibit significant decrease from control, while flies exposed to antioxcidants exhibit a significant increase. Statistical analysis was performed using one-way ANOVA followed by post-hoc Turkey's test with ** p 0.01, ***p 0.001 and ****p 0.0001.

Group P1 – Fertility Assay:

In the fertility assay, flies in the antioxidant groups produced more larvae than the control group, with the quercetin condition producing the highest number of larvae, followed by melatonin and NAC. On the other hand, files in NPS groups predictably produced fewer larvae, with gold having the largest detrimental effect of reducing the average larvae count to 64.3 (Figure 4).

Group P2 – Mobility Assay:

The second experiment, done using the parent generation, is named P2. In this experiment, flies are exposed to different combinations of nanoparticles (Table 2). Flies in the different combinations performed similarly in the mobility assay. When interpreted in conjunction with the P1 data, we observed that despite being the most detrimental nanoparticle when exposed individually, the effect of gold was greatly mitigated by the antioxidants. The antioxidant that was most effective in counteracting the oxidative stress caused by the AuNPs was melatonin, restoring flies' locomotion to even above the control group during the initial days of the experiment. Following melatonin, the NAC, and Quercetin groups also significantly decreased the mobility defect from oxidative stress that AuNPs caused (Figures 5, 6).

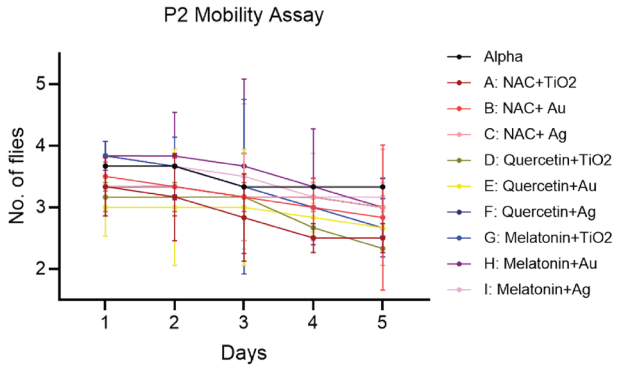

Figure 5: Mobility of parent flies exposed to combinations of compounds. All combinations demonstrate a non-significant difference, suggesting a revearsal effect from the antioxidants on the detrimental effect from exposure to nanoparticles.

TiO2 had similar results with some notable discrepancies: Melatonin rescued some locomotion defects, but overall mobility was still lower than the control group. NAC and quercetin were similar in terms of mitigation, with NAC still being more effective. We also observed that while the effect of TiO2 is mitigated by the antioxidants, the mitigation effect was less than what was observed in AuNPS (Figure 5,7).

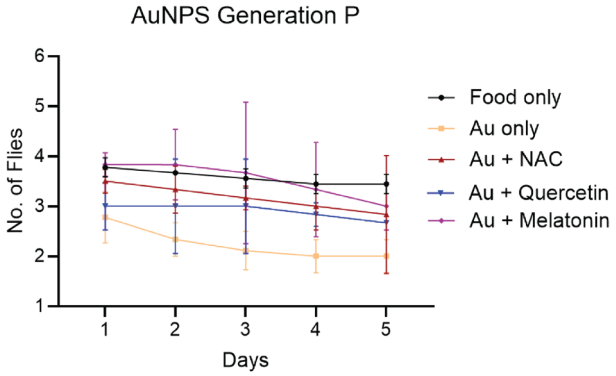

Figure 6: Mobility assay of parent flies exposed to AuNPs in combination with different antioxidants. The AuNPs-only group showd the lowest mobility, while every other combination showed a non-significant difference from the control. This suggests a reversal effect from the antioxidants in mediating the effect of AuNPs.

The combination of AgNPs and antioxidant exposure yielded the most interesting results. While there was no noticeable mitigation from either quercetin or NAC, the addition of melatonin improved mobility to almost the same extent as the TiO2 group, mitigated by melatonin (Figures 7, 8). This suggests that melatonin might be able to counteract the effects of Silver due to its protective properties against neurotoxicity in addition to its antioxidant properties. We speculate that silver has a neurotoxic effect on *Drosophila melanogaster*, causing a decrease in fitness. This would also explain why quercetin consistently performed marginally better than NAC despite causing worse mobility in the individual conditions group, as quercetin has also been linked to ameliorating neurotoxic effects. This would also give an insight into the marginally worse performance of the antioxidants in counteracting TiO2, which

has a verified neurotoxic effect as well, due to triggering neuroinflammation.

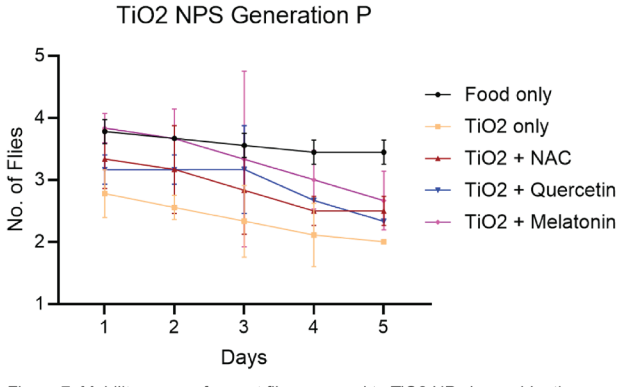

Figure 7: Mobility assay of parent flies exposed to TiO2 NPs in combination with different antioxidants. The TiO2 NPs-only group showed the lowest mobility, while every other combination showed a non-significant difference from the control. This suggests a reversal effect from the antioxidants in mediating the effect of TiO2 NPs.

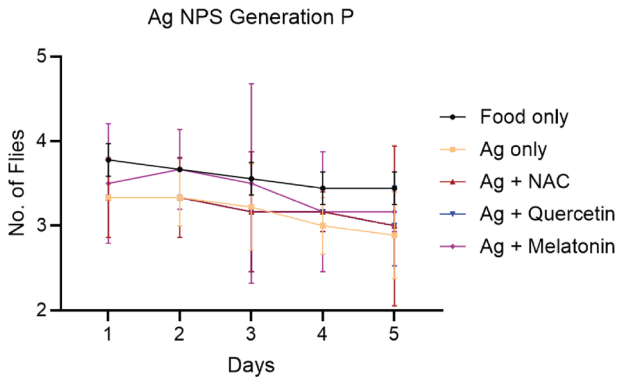

Figure 8: Mobility assay of parent flies exposed to Ag NPs in combination with different antioxidants. The Ag NPs-only and the Ag + NAC combination group showed the lowest mobility, while every other group showing a non-significant difference from the control. This suggests a reversal effect from the antioxidants, while also demonstrating silver's resistance.

Group P2 – Fertility Assay:

In the fertility analysis of P2, we observed that all Groups A-H showed lower larval count than the control. Group I (AgNPS + Melatonin) was the only group with a larval count of 83.5, close to the control group larval count of 84. Compared to other oxidants (Groups C and F), melatonin is potentially more effective in counteracting the negative effects of AgNPs. Other groups performed similarly, with larval count lower than the control group. Groups treated with melatonin (Groups G, H, I) generally showed a higher larval count (Figure 9).

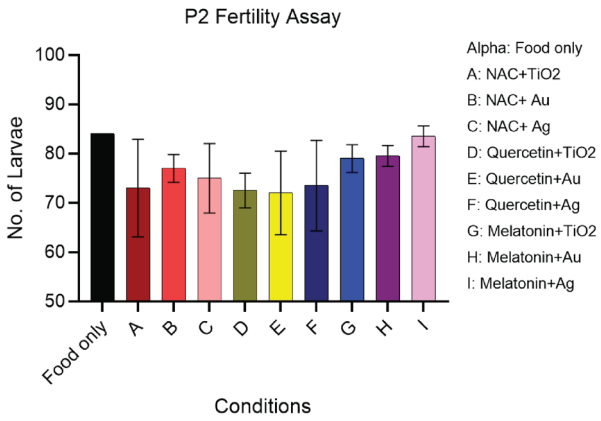

Figure 9: Mobility assay of parent flies exposed to different chemical combinations. All combinations demonstrate a non-significant difference compared to the control, indicating a reversal effect from the antioxidants. Statistical analysis was performed using one-way ANOVA followed by post-hoc Tukey's test.

Group F1 – Mobility Assay:

In the F1 group, we examined the first generation of offspring for mobility effects after the parent generations had been exposed to the individual compound. Offspring flies from conditions containing AuNPS, AgNPS, and TiO2 continued to show reduced mobility. Offspring from the AgNPS condition have the lowest mobility. On the other hand, offspring flies from Quercetin, melatonin, and NAC showed mobility similar to the control group (Figure 10).

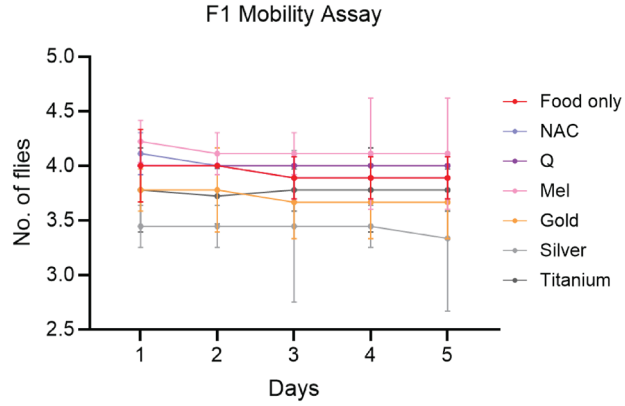

Figure 10: Mobility assay of spring flies from parent flies exposed to individual compounds. Offspring of parents exposed to melatonin, gold, and titanium showed significant differences from control. Silver also demonstrated a significant decrease from the control.

Group F1 – Fertility Assay:

In the F1 group, offspring flies from the quercetin and melatonin conditions showed a noticeable increase in larval count compared to the control group. On the other hand, NAC and TiO2 conditions have larvae count similar to the control group. The largest decrease in larval counts was observed in the offspring from the AuNPS and AgNPS conditions. This suggests to us that the detrimental impact of AgNPs and AuNPs is exerted transgenerationally on the fertility capability of the offspring (Figure 11). Together with the mobility assay data, AgNPs and AuNPs seem to exert detrimental transgenerational effects on both the fertility and mobility of *Drosophila*

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melanogaster (Figures 10, 11). Melatonin and quercetin, on the other hand, also have a transgenerational impact, with melatonin affecting fertility and mobility, and quercetin impacting mainly fertility (Figures 10, 11).

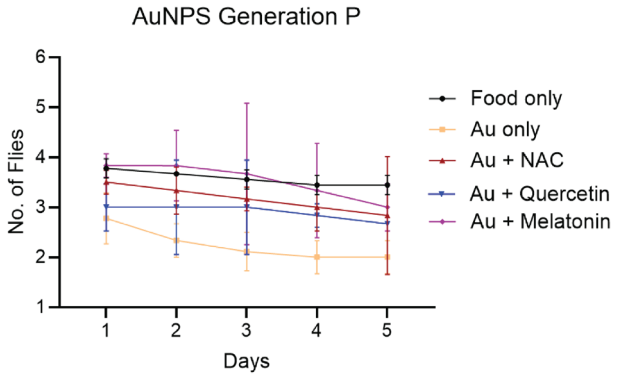

Figure 11: Fertility assay of offspring flies from parent flies exposed to individual compounds. Flies from parents exposed to nanoparticles exhibit a significant decrease from control, except for TiO2 NPs. Flies from parents exposed to antioxidants exhibited a significant increase from the control, except for NAC. Statistical anlaysis was performed using one way ANOVA followed by post-hoc Tukey's test with ***p<0.0001.

Group F2 – Mobility Assay:

In the F2 group, we examined the first generation of offspring for mobility effects after the parent generations had been exposed to combinations of different compounds (Figure 12). The F2 group yielded interesting and exciting results. While the Au nanoparticles performed lower in the F1 group, all groups treated with antioxidants performed similarly to the control group in mobility assays (Figure 13). For both AuNPs and TiO2 nanoparticles conditions, offspring flies exhibit a higher performance in mobility with all three antioxidants (Figures 14, 15). Despite not demonstrating a clear transgenerational impact on F2 flies, TiO2 still scored lower in all categories when compared to the antioxidant-treated vials in F2. AgNPs were the only nanoparticles that seemed to display persistent effects despite being treated with antioxidants. Both the quercetin and the NAC-treated silver groups showed no discernible improvements in mobility. However, the melatonin-treated groups did score very close to the control group in mobility assessments. These results are significant because they suggest a clear reversal of the transgenerational damage caused by every nanoparticle. This data suggests that the antioxidants tested can reverse the transgenerational damage caused by both titanium and gold nanoparticles, while melatonin appears to have the unique ability to counteract the effects of silver nanoparticles, likely due to its research link to counteracting neurotoxic effects (Figures 12, 13, 14, 15).

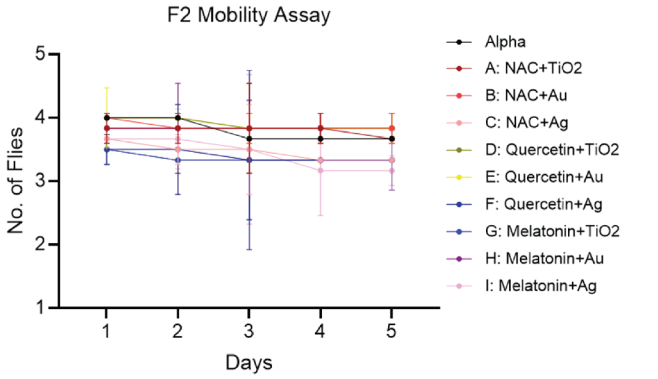

Figure 12: Mobility assay of offspring flies from parent flies exposed to combinations of chemicals. All combinations except C, F, and G demonstrate a non-significant difference compared to control, suggesting a reversal effect from the antioxidants. Conditions C, F, and G showed a significant decrease from the control. Statistical analysis was performed using one-way ANOVA followed by post-hoc Tukey's test.

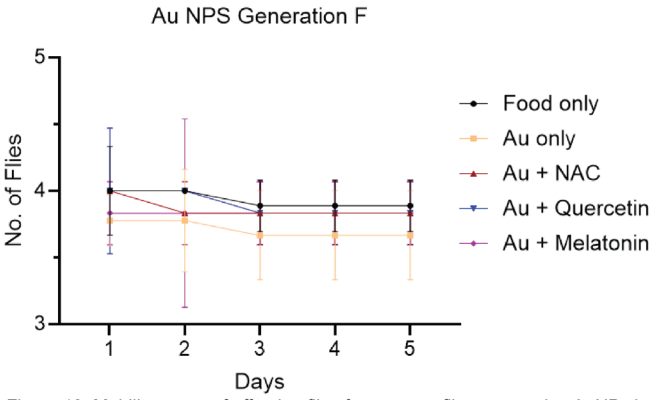

Figure 13: Mobility assay of offspring flies from parent flies exposed to AuNPs in combination with different antioxidants. The AuNPs-only group showed the lowest mobility, with other combinations showed a non-significant difference from the control. This suggests a reversal effect from the antioxidants.

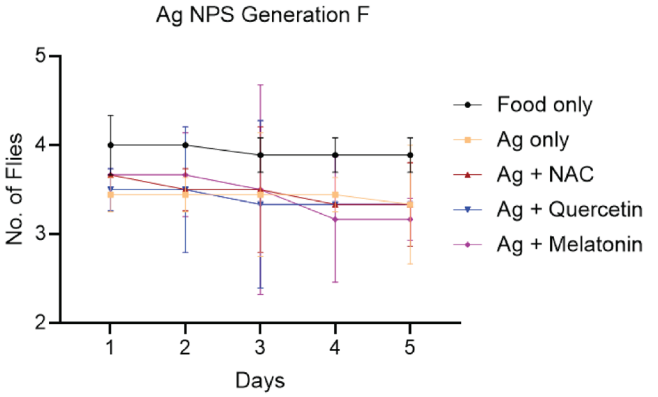

Figure 14: Mobility assay of offspring flies from flies exposed to AgNPs in combination with different antioxidants. The AgNPs-only group showed the lowest mobility. Other combinations except for Melatonin showed a significant decrease from the control.

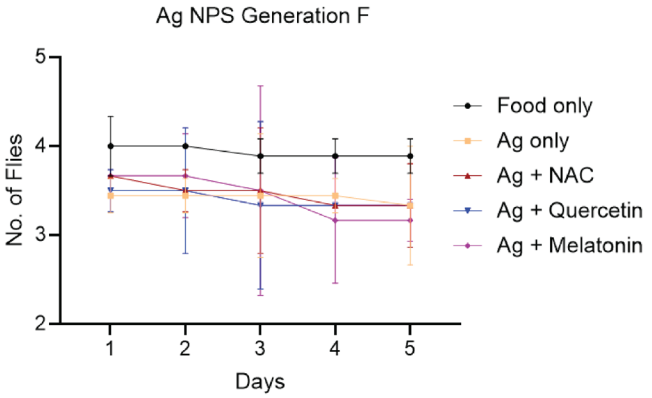

Figure 15: Mobility assay of offspring flies from parent flies exposed to TiO2 NPs in combination with different antioxidants. No combinations showed any significant difference from the control.

Group F2 – Fertility Assay:

In the F2 group, offspring flies from the TiO2 groups, when combined with NAC and Melatonin (Figure 16, Conditions A and G), showed a recovery of fertility, while the combination with Quercetin did not (Figure 16, Condition D). The offspring flies from the AuNPs group in combination with NAC and quercetin (Figure 16, Conditions B and E) exhibited recovered fertility compared to the control group. AuNPs in combination with melatonin did not show a recovery in fertility rate (Figure 16, Condition H). Lastly, offspring flies from AgNPs, when in combination with quercetin and NAC (Figure 16, Conditions C, F), did not show any recovery of fertility. However, the exposure of melatonin in combination with AgNPs results in a fertility rate close to that of the control group.

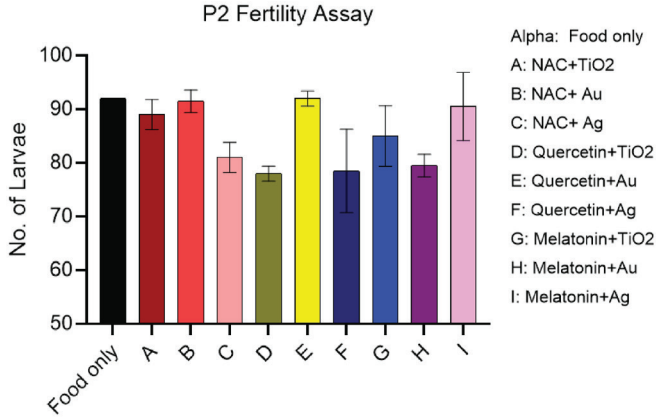

Figure 16: Fertility assay of offspring flieds from parent flies exposed to different chemical combinations. All combinations demonstrate a nonsignificant difference compared to the control, suggesting a reversal effect from the antioxidants.

Results and Data Analysis

In our studies, we observed that each nanoparticle has exerted a detrimental effect on both the mobility and fertility of Drosophila melanogaster. Residual effect was still observed in the next generation on mobility, and to a lesser extent, fertility. Interestingly, the reverse was observed on antioxidant compounds. Antioxidants improved both mobility and fertility in the parent flies, and quercetin and melatonin continued to have that beneficial effect on the offspring's fertility.

When nanoparticles and antioxidants were studied in combination, our data strongly suggest that AgNPs have the strongest detrimental impact on mobility and fertility. AuNPs and TiO2 also exert a detrimental effect, but not to the extent of AgNPs. NAC and quercetin were unable to reverse the detrimental effect in both mobility and fertility assays, even though they were able to do so when combined with TiO2 and AuNPs. Melatonin was the only antioxidant that exhibited a reversal effect on the negative impact of AgNPs.

Data was analyzed for statistical significance using one-way ANOVA of the trial averages of each replicate. Each replicate, using the mean across the trials, was assessed on its significance from the control groups using Tukey's post-hoc test. For analyzing the fertility assays, due to the single-time measurement nature of that assay, all raw data were utilized in the data analysis. This data analysis was conducted using a significance level (α) of 0.05.

The data analysis also brought irregularities in the parent generations' results forward, such as silver performing unusually high in the P1 mobility assay than what would be expected when compared to its results in the other assay. Error bars were calculated using n=5.

The results of this data analysis primarily supported our interpretation of the results. Individual conditions demonstrated significant differences from the control for all of the nanoparticles across both types of assays, while the positive effect of the antioxidants was primarily insignificant in the mobility assays, except for melatonin, which appeared statistically significant in both mobility assays. All chemicals tested were significant in at least one of the two fertility assays. Conversely to the individual conditions, the combined condition assays demonstrated little significant difference from the control in mobility, and none in either generation for fertility. The outliers in mobility were groups C (Silver + NAC), F (Silver + Quercetin), and G (TiO2 + Melatonin), as discussed earlier. These results verify our hypothesis and previous postulations as they indicate a clear reversal effect from the antioxidants.

Limitations and Further Experimentation:

These experiments were performed in an informal, at-home lab setting with experimental materials limited to commercially available resources. This experiment was conducted on a tight 7-week schedule and thus was limited to observing 2 generations of *Drosophila melanogaster*. While this setup allowed us to observe and measure the behavior of *Drosophila* flies, we were unable to perform any genetic or biochemical experiments to follow up on our observations. The lack of microgram-precise measuring tools also made it difficult to measure the exact concentrations of all of the vials. We improvised by reconstituting a concentrated solution before further serial dilution. Therefore, we need to keep in mind that there might be variations resulting from this technical issue. Finally, it is important to note that none of the tests are perfect measurements of the physical or reproductive health of *Drosophila melanogaster* and are subject to both human and experimental variation.

Nonetheless, our observations brought up some interesting questions for future experiments. Further experiments

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on this topic will include an expanded scope of nanoparticles and reversal compounds while also studying transgenerational impacts across more than 2 generations. If the scope of antioxidants tested were expanded, it would be interesting to test compounds reported to alleviate neurotoxic effects. Furthermore, our data raises interesting follow-up questions on whether *Drosophila* genetic variations would give flies an edge in survival: Are there any genetic mutations that would allow flies to resist nanoparticles' detrimental effect, or enhance the beneficial effect of antioxidants? Advancing this topic into gene analysis can give us an insight into genetic variation in flies, and the results are potentially translatable to human health. Finally, our data identified a few compounds with a significant impact on *Drosophila* health, such as silver nanoparticles and melatonin. Further studies can include biochemical assays to identify the exact cellular pathways through which these compounds exert their effect.

Conclusion

This study further verifies the significant impact that the tested nanoparticles exhibit on the mobility and fertility of Drosophila melanogaster, as well as the potential for mitigating these effects exhibited by the tested antioxidants. Gold, silver, and titanium dioxide nanoparticles exhibited detrimental effects on both fertility and mobility, with Gold and silver demonstrating transgenerational impacts. The antioxidants: melatonin, quercetin, and NAC all demonstrated a positive effect on these metrics, with melatonin demonstrating these effects transgenerationally.

Notably, the results of the second generation of flies suggest that treatment with the tested antioxidants can reverse transgenerational impacts in the nanoparticles tested, with melatonin being especially effective, as it was the only antioxidant to counteract the damaging effects of every nanoparticle tested. These findings highlight the potential for antioxidants to counteract the long-term biological consequences of nanoparticle exposure. However, considering the limitations of this study, including an informal lab setting, lack of genetic analysis, and restricted generational scope, further research is necessary. Future research might expand the range of nanoparticles tested, assess genetic influences, and expand the analysis to cover more generations. These studies would help to better understand the long-term implications of nanoparticle exposure.

In summary, this research has offered some valuable insights into the interplay between oxidative stress and neurotoxicity in the physical and reproductive health of *Drosophila melanogaster*, but given its informal nature, it is my personal opinion that further and more thorough research would be beneficial in investigating how to mitigate transgenerational toxicity in living organisms.

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