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Research Paper

Ingestion of Gold Nanoparticles (AuNPs) Affects Survival in *Drosophila* in A Dose-Dependent Manner

Biological Science

KEYWORDS : Gold nanoparticles (AuNPs), Drosophila melanogaster, Fertility, Survival

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ABSTRACT

The enormous usage of gold nanoparticles (AuNPs) in wide array of applications such as environmental, engineering, industrial, biology and medical sciences are raising concerns about their potential effect on human health. Therefore, to assess its safety issues, dose-dependent impact of AuNP on different aspects like survival, behavior and fertility was investigated using Drosophila as a model system. We found that ingestion of AuNP at larval stage impairs survival of the progeny in a dose-dependent manner. However, their behavioral activities such as crawling, climbing and fertility remains unaffected even at a higher dose. Our results clearly suggest that exposure to higher dose of AuNP can negatively affect the survival of the progeny.

1. Introduction

The modern science of nanoparticle synthesis has fascinated a wide area of disciplines such as chemistry, biology, medicine etc. Particles in the size of nanometers are reported to be more advantageous over the bulk counterparts as it offers higher surface area to volume ratio (Nam, et al., 2008). In ancient times, the lustrous glazing artworks on glass were made using colloidal gold; besides that gold is also known to be utilized for various medical purposes (Hayat et al., 1989). Due to extraordinary uniqueness of gold, now a day's gold nanoparticles are being increasingly synthesized and used as drug carriers (Everts, et al., 2006; Gibson, et al., 2007; Paciotti, et al., 2004) in diagnostics and therapeutic purposes (Goodman, et al., 2004; Nel, et al., 2006).

Till date, the guidelines for AuNP toxicity and health related issues have not been defined. Therefore, it is very important to understand their potential toxicological aspect and dose of AuNP that can be hazardous to human health, particularly in an in vivo model system. A very limited in vitro study using different cell lines such as human leukemia cells (Connor, et al,. 2005), human dermal fibroblast (Qu, et al., 2009), optical cells (Hartono, et al., 2010), human prostate carcinoma cells (Arnid, et al., 2010) and human liver cell lines (Gao, et al., 2011) have been done which suggests that AuNP has the potential to induce oxidative damage to the cells. It is reported to accumulate in different organs such as liver and spleen of rat (Balasubramanian, et al., 2010; De Jong, et al., 2008) and causes acute inflammation and apoptosis (Cho, et al., 2009). Moreover, Lasagna et al. have shown that in vivo AuNPs can cross the blood brain barrier and accumulate in neural tissues. Recently, in another in vivo study using Drosophila, gold nanoparticles have been shown to induce aberrant phenotypes by causing genotoxicity (Vecchio, et al., 2012). All these reports on AuNP strongly suggest that AuNP that is being used routinely in day to day life could be of serious concern due to their potential to damage the tissues. Therefore, it is very important to understand the impactof these nanoparticles in the living system and to define the dose that is safe for it to be incorporated in consumer goods including medicine etc.

We aimed towards understanding the dosage effect of AuNP in an in vivo system using Drosophila as a model organism. The dose-dependent impact of AuNP was investigated on different aspects such as survival, behavior and fertility by feeding Drosophila with different doses of AuNP. We found that AuNP affects survival of the larvae if supplemented early during first larval instar.

2. Materials and methods

2.1 Fly strain and culture

Wild-type D. melanogaster were raised at 25±1ºC.on standard cornmeal food containing corn flour, yeast, sugar, agar-agar and propionic acid

2.2 Drosophila food preparation and feeding with gold nanoparticles

Gold nanoparticles (Sigma Aldrich, give catalog #) of 30 nm in size were used for this study. AuNP was added and mixed thoroughly into the partially cooled fly food to obtain the final concentration of 50pM and 150pM. Finally, after mixing, the food was poured into culture vials.

For analyzing the effect of AuNP ingestion on survival, behavior and fertility; larval feeding was done by exposing the eggs laid by parent flies to different doses of gold nanoparticles

2.3 Crawling assay

The locomotor activity of wandering third instar larvae was monitored by allowing them to move in a groove (2 mm wide, 30 mm long and 5 mm deep) made on a petridish containing 3.3% agar. The distance covered by treated and untreated larvae in 30 seconds was recorded.

2.4 Developmental assessment and survival

Parent flies were allowed to lay eggs and thereafter, 75 eggs were transferred in vials containing food supplemented with different concentrations of AuNP. The pupation ability was determined by counting the number of pre-pupae and pupae formed out of 75 eggs. Similarly, adult eclosion rate was determined by scoring the flies emerged out of their pupal case.

2.5 Climbing assay

After giving larval treatment with different doses of AuNP, the flies emerging from different treatment conditions were monitored for their vertical climbing ability. 10 flies were set up in two replicate vials per treatment. The number of flies that climbed 10 centimeters in 15 seconds in three repeats per vial was recorded on Day1 and Day7 post Eclosion.

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2.6 Analysis of egg laying capability and fecundity

Virgin female and male flies were collected and housed in a chamber for 2 days to allow mating before monitoring their egg laying capability. Thereafter, mated parents were allowed to lay eggs on standard fly food for four hours at 25±1·C. A set of 50 mated female flies were made in the replicates of two and the number of eggs laid within four hours of oviposition were counted to determine their rate of egg laying.

The fecundity was determined by counting the number of 1st instar larvae hatching from the eggs laid by treated and untreated female flies.

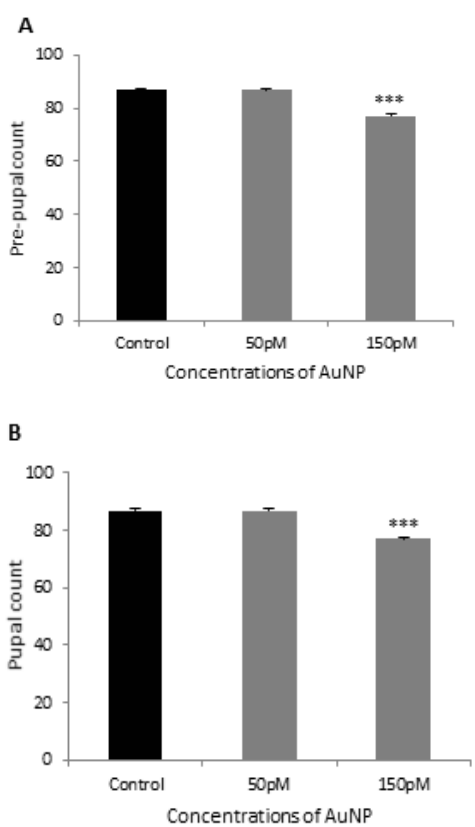
2.7 Statistics

All the AuNP treated conditions at different doses were compared with untreated condition (control) using Student's t-test. (*=P<0.05, **= P<0.01, ***=P<0.001).

3. Results and Discussion

3.1 AuNP exposure at larval stage impairs survival of the progeny in dose-dependent manner

For monitoring the percentage of survival, 75 eggs were transferred from the parents in each vial containing food supplemented with different doses of AuNP i.e. control, 50pM and 150 pM. Number of larvae that could reach upto pre-pupal and then to adult were counted, The percentage of larvae that could reach the pre-pupal (Figure 1A) and pupal stage (Figure 1B) were significantly lower at 150pM of AuNP as compared to the control (p<0.001). Further, the adult eclosion rate (Figure 1C) was also negatively affected at 150pM of AuNP (p<0.001); however 50pM of AuNP did not show any negative effect on both pupal formation and adult survival rate. Our observations suggest that ingestion of AuNP at 150pM results in larval death which causes reduction in the survival of progeny.



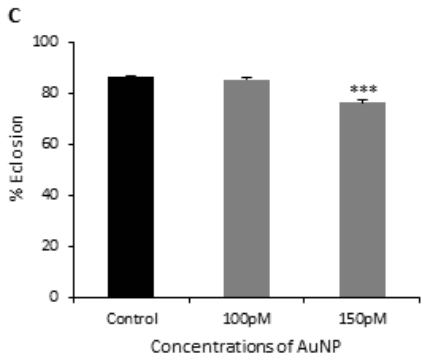


Fig1. Impact of AuNP exposure on survival of progeny. A. The pre-pupal, B. pupal formation and C. adult eclosion was determined in the presence of AuNP treatment during larval stage. ***, P<0.001

3.1 Dietary intake of AuNP has no negative effect on behavior of Drosophila melanogaster

In order to understand the effect of AuNP on behavior, larval crawling and adult climbing ability was monitored. We found that feeding larvae with food supplemented with different concentrations of AuNP did not affect its crawling ability (Figure 2A). Similarly, the adult climbing ability was monitored on day 1 and 7 post eclosion. We found no difference in the vertical movement of the flies of both control as well as AuNP treated flies (Figure 2B). Our results show that all the doses of AuNP administered during the larval development did not compromise the behavioral activities of the progeny.

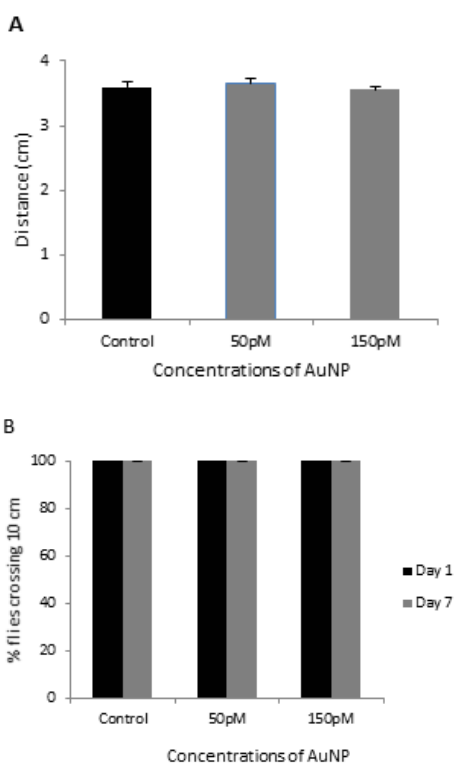


Fig2. Behavioral activity in flies raised on AuNP supplemented food. A. The crawling ability and B. climbing behavior treated and untreated progeny flies.

3.3 Effect of AuNP on female fertility

The effect of AuNP treatment on fertility was analyzed by allowing the newly emerged male and female flies from

their respective treatment conditions to mate for 2 days. Thereafter, to determine the egg laying capability, the mated female flies were allowed to lay eggs for 4 hours. The numbers of eggs scored in all the treatment conditions were nearly the same as in control. Our results clearly indicate that AuNP at 50pM and 150pM doses is safe and does not affect the reproductive health of female flies (Figure 3A).

Further, since the egg production rate was unaffected, we were interested in investigating, if fecundity of these AuNP treated flies gets compromised or not. The percentage of viable F2 generation offspring was determined by counting the number of 1st instar larvae hatching out from the eggs laid by treated and untreated female flies. We found that the percentage of L1 hatching remain unaffected at different doses of AuNP suggesting that viability of F2 generation does not get influenced by AuNP exposure at larval stage (Figure 3B).

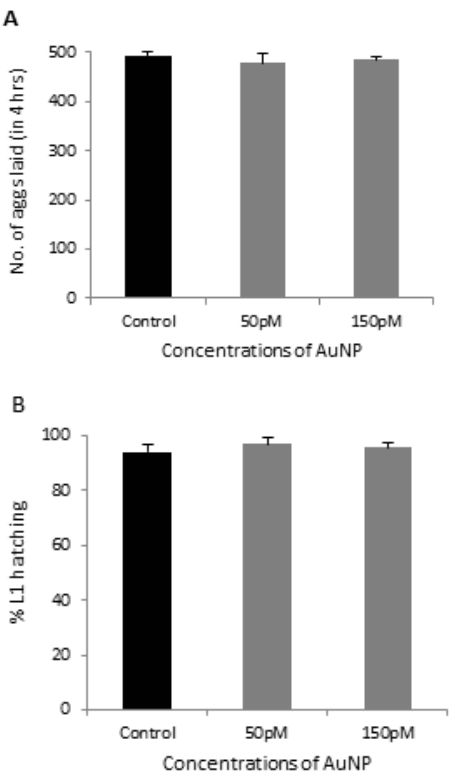


Fig 3: Fertility of the progeny flies reared on AuNP supplemented food. A. The egg laying capability of treated and untreated female flies B. L1 hatching rate of F2 generation.

Conclusion

Our results strongly employ that AuNP exposure during larval development impairs the survival of the progeny at a higher dose by causing larval death. However, it has no negative influence on the behavior and fertility of the flies reared of AuNP supplemented food, when compared with the control flies. Additionally, the fecundity of F1 generation flies clearly suggests that the viability of the F2 generation progeny remains unaffected and the impact of AuNP ingestion is not being carried forward to the future generations.

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