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Effects of neodymium magnetic exposure on fertility indicators in *Drosophila melanogaster*

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ABSTRACT
Current investigation reveals the effects of rare earth element (REE) as lanthanide metal, neodymium (Nd_2^{+6}) on the reproduction of Drosophila melanogaster in the behavioral pattern i.e., copulation duration, fecundity and fertility are studied by the exposure of lethal concentrations. The REE lanthanide metal was added to a wheat cream agar media in graded doses (0.02mg, 0.03mg, 0.04mg). D. melanogaster species were cultivated under optimal conditions in a standard wheat cream agar media. Third instar larvae of the flies were chosen for the ingestion treatment due to their hyper activity like boring, crawling, and voracious feeding. Results showed that on exposure of Nd_2^{+6} graded doses of concentration to treated versus the untreated control showed decreased fecundity and fertility significantly ($p<0.05$), whereas the copulation duration was increased in the treated flies than that of the control with different crosses. The toxicant showed a significant decrease in fecundity and fertility when compared to control group, also exhibited increasing level of copulation duration without affecting their fecundity in each treated group significantly ($p<0.05$). On treating with graded doses of Nd_2^{+6} metal for the reciprocal crosses has revealed that variation in concentration continuous exposure can affect the physical fitness of the species whether it's a copulation duration, fecundity and fertility.
Keywords: *Drosophila melanogaster, Neodymium, Reproduction, Fecundity, Fertility*

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INTRODUCTION

The neodymium chloride ($NdCl_3$) studies of pharmacology and toxicology have been reported for delayed acute toxicity in higher animals as like as symptoms of those seen similar with other rare earth element (REE) series. Chronic oral ingestion of neodymium chloride for 12 weeks in a graded concentration at the three dose levels exhibited no change in the hematology parameters and histopathological damage in the studied internal organs [1].

Inhalation of neodymium oxide induces the various respiratory diseases, such as lung inflammation, but the associated mechanisms are still unclear. Such studies are needed to explore the role and mechanisms of molecular level in circular RNA causes behind the exposure and induced airway inflammation. Study showed that treating neodymium oxide to human bronchial epithelial (16HBE) cells caused an inflammatory issue and to response was upregulated the gene expression of interleukin-8 (IL-8) and interleukin-1 beta (IL-1Î²) [2].

The effect of Nd_2^{+6} nanopolymorphs were studied extensively to induce various biological issues in that particularly on angiogenic process was observed and they attributed directly with the ability to modulate redox signaling. This was observed to be via PKM2-NOX4 signaling pathways. Further the results demonstrated that ROS generated by cuboid and rod shaped nanopolymorphs activated the pro-angiogenic factors namely VE-cadherin, HIF 1α, VEGF and VEGFR-2 to facilitate the angiogenic process [3]. The trivalent cations lanthanum, neodymium and europium were found to have a weak dose-dependent antinociceptive effect as measured on hot plate and tail nick following administration into the lumbar subarachnoid space of rats implanted with chronic intrathecal catheters [4].

Recent research thoroughly demonstrates that the efforts in trying to know the neural mechanism which includes instinct behaviors such as mating and fighting, it has driven by the advanced techniques to monitor and manipulate the neuronal activity [5-7]. *Drosophila* has great potential as a model system for studying toxic effects because they have metallothionein similar to those of mammals [8]. *Drosophila* also

has a lot of similarities in the developmental pathways, signalling pathways as well as some protein-coding pathways, say for example the Notch signalling pathway in the embryonic development of the nervous system in fruit flies and humans are similar. [9] The fruit fly is a convenient system to address this question since many aspects of metal homeostasis are conserved between flies and humans [10]. It is an ideal model to conduct preliminary studies like fitness analysis. Net fitness comprises various components such as viability, female fecundity, male mating ability, developmental time, longevity, etc. [11] Fitness plays a central role in evolutionary biology which has inspired numerous treatments of this concept from both theoretical and experimental points of view experimentation with *Drosophila* has been quite diverse, ranging from the measurement of a single component of fitness in individual flies to estimates of overall fitness based on the long-term reproductive success [12]. Since fitness is majorly regulated by dietary and environmental factors, it is susceptible to changes associated. Stress acts as the key factor in reducing fitness of an organism and heavy metal intoxication is one of them [13]. *Drosophila* facilitates the easy manipulation and analysis of this parameter thereby providing us the insights that can be utilized in the process of understanding the complex homeostatic mechanisms of human body.

D. melanogaster is a complex multi-cellular organism in which many aspects of development and behaviour parallel those in human beings. They are small have a life cycle of less than two weeks (short generation time) and grown on simple media, single male and female can produce more than 100 progenies (high reproductive rate), and ability to perform large-scale genetic and/or compound screens, which cannot be developed in other higher animal models due to cost, time and legal or ethical boundaries and issues. *Drosophila* sps exhibit morphologically similar cosmopolitan and human commensal species, but they differ in traits such as courtship behavior, genital morphology, ecophysiology, DNA and protein polymorphism [14]. In view of these, a study has been conducted on life history traits in a model organism *Drosophila melanogaster* to evaluate the effect of Nd_2^{+6} toxicity on fitness of flies upon exposure to graded concentration.

MATERIAL AND METHODS

Collection of Flies

The stocks were obtained from the National *Drosophila* stock centre, University of Mysore, India. The fly stocks were routinely cultured in standard wheat cream agar medium in un crowded condition at 22 ± 10 ^{o}C (rearing temperature), 12 : 12 hours photoperiods and a relative humidity of 70%. The test flies were cultured in wheat cream agar media supplemented with graded concentrations of Nd_2^{+6} (i.e., 0.02mg, 0.03mg and 0.04mg respectively).

Virgin females and unmated males were collected and maintained separately for 5 days in order to age and then transferred to vials containing media alongside a control. The rare earth metal was added to a wheat cream agar media in graded doses; (Nd_2^{+6} 0.02mg, 0.03mg, 0.04mg). The control cultures were maintained on the same diet without addition of rare earth element metal. Media (5ml) was placed in a 25 x100 mm sample tubes and a pair of flies was transferred to each vial. Likewise, 3 successive transfers were made into fresh food containing metal once in two days for the above said species of *Drosophila.*

Drosophila melanogaster species were cultivated under optimal conditions in a standard wheat cream agar media. Third instar larvae of the flies were chosen for the ingestion treatment due to their active crawling, boring and vigorous feeding. The control groups were cultivated in the standard medium, while the treated larvae were cultivated in a research medium, which consisted of standard media and Nd_2^{+6} metal. Experiment was conducted in triplets and data was recorded every 24h for 4 days.

Copulation Duration

Courtship duration (time from introduction of male and female together into the mating chamber and until the orientation of male towards the female) and copulation duration (time taken from initiation to termination of copulation of each pair) were recorded. A minimum of 30 pairs involving each cross were observed. Mating propensity was analysed for four different sets (crosses); untreated male with untreated female (C), treated male with untreated female (T1), untreated male with treated female (T2) and treated male with treated female (T3) for each species. A pair of flies was aspirated into empty glass vials to avoid etherisation. The mating activity was observed for 60 minutes. The pairs which do not mate within a stipulated time of 60 minutes were discarded [15].

Fecundity and Fertility

Virgin females and unmated males of *Drosophila melanogaster* were isolated, collected and reared separately on normal wheat cream agar media. Further these flies were fed on wheat cream agar media with different doses of REE metal along with control flies for three days. Four sets of crosses were made using thirty pairs of flies for each cross, facilitating single pair mating (each pair in a separate vial).

These crosses include-

1. Untreated male x Untreated female (Control-C).
2. Treated male x Untreated female (T1).
3. Untreated male x Treated female (T2).
4. Treated male x Treated female (T3).

A total of 120 pairs of flies were used to assess reproductive fitness (fecundity and fertility) [16].

Statistical Analysis

Data for fitness parameters was analyzed using SPSS 15.0 software with $p<0.05$ taken to indicate the significance statistically [17]. The effect of Nd_2^{+6} metal was assessed by One way Analysis of Variance (ANOVA) followed by Tukey's HSD test for post- hoc comparisons.

RESULTS

In the current investigation, exposure of Nd_2^{+6} metal is supporting for the growth and development of fruit flies, in environment such metal oxides are abundantly available and may be beneficial for the organism's ecological conditions and also interplay between physiology and environment for the sustainability.

Effect of Nd_2^{+6} Metal on Copulation Duration of *Drosophila melanogaster*

The mean copulation duration increased in T1 (Untreated ♀× treated♂) at 0.02 concentration and reduced in 0.03 and 0.04 with that of control, while T2 (Treated ♀× Untreated♂) showed increased copulation duration with increase in the doses with that of the control and T3 (Treated ♀× Treated♂) showed reduction in all the graded doses (i.e., 0.02, 0.03 and 0.04mg/ml) significantly ($p<0.05$) of Nd_2^{+6} compared to that of control as shown in the Figure 1. The significant reduction is observed between groups with that of the control in *D. melanogaster* as shown in Table 1.

Effect of Nd_2^{+6} Metal on Fecundity of *Drosophila melanogaster*

The mean fecundity was reduced in T1 (Untreated ♀× treated♂), T2 (Treated ♀× Untreated♂) and T3 (Treated ♀× Treated♂) on exposure to different concentrations (i.e., 0.02, 0.03 and 0.04mg/ml) of Nd_2^{+6} as shown in the Figure 2. The significant reduction is observed between groups with that of the control and was highly significant in 0.04mg/ml in *Drosophila simulans* as shown in Table 1.

Effect of Nd_2^{+6} Metal on Fertility of *Drosophila melanogaster*

The mean fertility was reduced in T1 (Untreated ♀× treated♂), T2 (Treated ♀× Untreated♂) and T3(Treated ♀× Treated♂) on exposure to different concentrations (i.e., 0.02,0.03 and 0.04mg/ml) of Nd_2^{+6} as shown in the Figure 3. The significant reduction is observed between groups with that of the control and was highly significant in 0.04mg/ml in *Drosophila melanogaster* as shown in Table 1.

DISCUSSION

Studies on courtship and developmental behavior in experiments have stepped on two factors of transcriptions to influence the sex-determination in Drosophila in the hierarchy of fruitless (fru) and double sex (dsx) gene. These factors demonstrate it as sex-specific physiology and neural circuitry [18]. In previous findings on fru and dsx factors regulate during the time of courtship, expressed it invariably and analysed behavior in males and females which shows mutations in the respective loci [18]. These genes were regulate the sexual behavior and specify courtship behaviour as per the environmental, nutritional and spatial patterns under the influence of nervous system, which establish to determine candidate cellular nature of the neuronal network [18- 20].

The exposure of rare earth element (REE) exhibit many changes in the liver morphology. After REE enter in to the liver, they will interact with many proteins and other biomolecules in the live cells. REE also involve in enzyme activities to interfere and elevate the liver physiological functions through the biomolecules [21]. The mating behaviour and reproductive process in *D. melanogaster* is well studied and provides a useful background. Mating is an important mechanism to propagate and enrich the species population. Behaviours controlling the propensity to mate can have large effects on fitness [22]. Mating behaviour of *Drosophila* exhibit very systematically, which are planned specific orientation, movements possess several communications and which are resulted sequentially [23]. These factors influence for the variation in copulation duration, they are very complex and species-specific traits in *Drosophila* [24].

Behaviour of adult flies involved with fine motor coordination for courtship and mating was used to examine aspects of behaviours relevant to human diseases [25]. In view of this, the courtship duration and copulation duration among untreated and treated flies (reciprocal crosses) were studied on different species of *Drosophila*. Mating activity is correlated with fitness in species of *Drosophila*. There is considerable variation in courtship and copulation duration among the species of *Drosophila*. The mating propensities of flies performing a particular behaviour at any given concentration level were significantly different. To determine if the observed impairments in mating behaviour in Nd_2^{+6} metal treated flies were due to dysfunction in the male, female, or both. Specifically, the performance of the treated male flies and

females (T3) were significantly reduced ($p<0.05$) compared to other group received lower concentration and control results of pairs (C, T1, T2). This phenomenon due to the variation in the dynamics and interval during the interaction of male and female also noticed when exposure of REE to one partner. When male and female partners were exposed (T3), they shown poor interest in reproductive pattern of behaviour like courtship and copulation activities. In reference to male flies exposure, they were not very poor interest and interaction with the females. Furthermore, exposure makes them separate in many instances where the females shown interest on the male but male refuse and stay away from them. When exposure of lanthanum oxide only to female, male exhibit maximum interest and vigorous attempt of courtship but females lack of interest on to mate with the male. A role of specific gene which influences it significantly to involve in pattern of courtship behaviour is fruitless (fru), in all the phase of mating process completely disrupted and depends on certain alleles which are expressed through the locus. [26]. The REE act as trivalent ion, Nd_2^{+6} metal was studied for their exposure efficacy on Escherichia coli growth rate, transformation in their physiology, and gene expression. The results of the Nd_2^{+6} various concentrations such as 50 to 150 µg/mL exhibited a change in endogenic and ectogenic metabolism, but also observed some defects by gene expression. Nd_2^{+6} metal at lower various concentrations such as 0.5 to 30 µg/mL significantly inhibit the E. coli from absorbing deoxyribonucleic acid (DNA) externally and decreasing their transformation efficiency [27]. REE exposure on mononuclear cells of human peripheral blood (PBMNCs) significantly increased telomerase activity and cell percentage in the cell cycle specifically S-phase and thereafter G2/M phase, but non-significant effect on the apoptotic cell rate in PBMNCs. Under lower concentrations, exposure of REE exhibited telomerase potential of PBMNCs when exposed to higher concentrations that of the group control, and also observed the non-significant effect in the apoptotic cell rate in the PBMNCs, but noted that the promotion of DNA replication (2n) and their percentage increased during the phase of G2/M- and S-phase cells. [28]. In the present work, the values of the mean minimum and maximum concentrations of neodymium on flies were found to be significant for fecundity and fertility affecting the rate of development in the species with that of the control in reference to all the reciprocal crosses of treated and untreated *Drosophila melanogaster* flies.

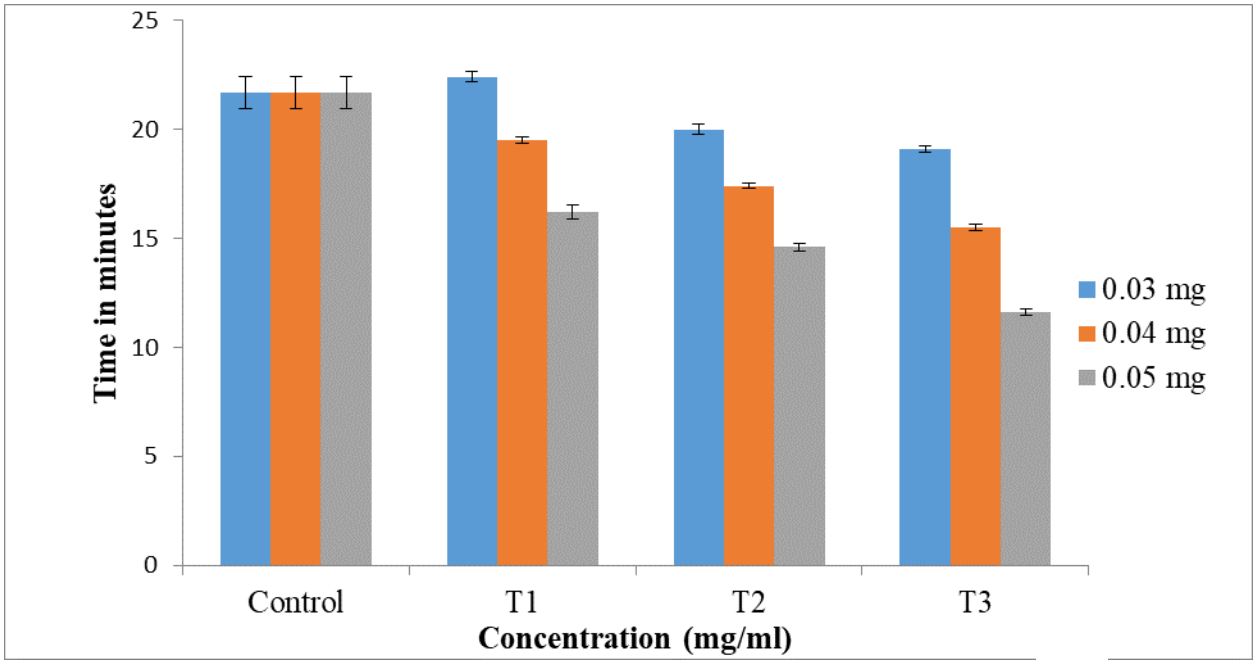

Fig 1. Mean (±SE) copulation duration of *Drosophila melanogaster* on exposure to Nd_2^{+6} metal

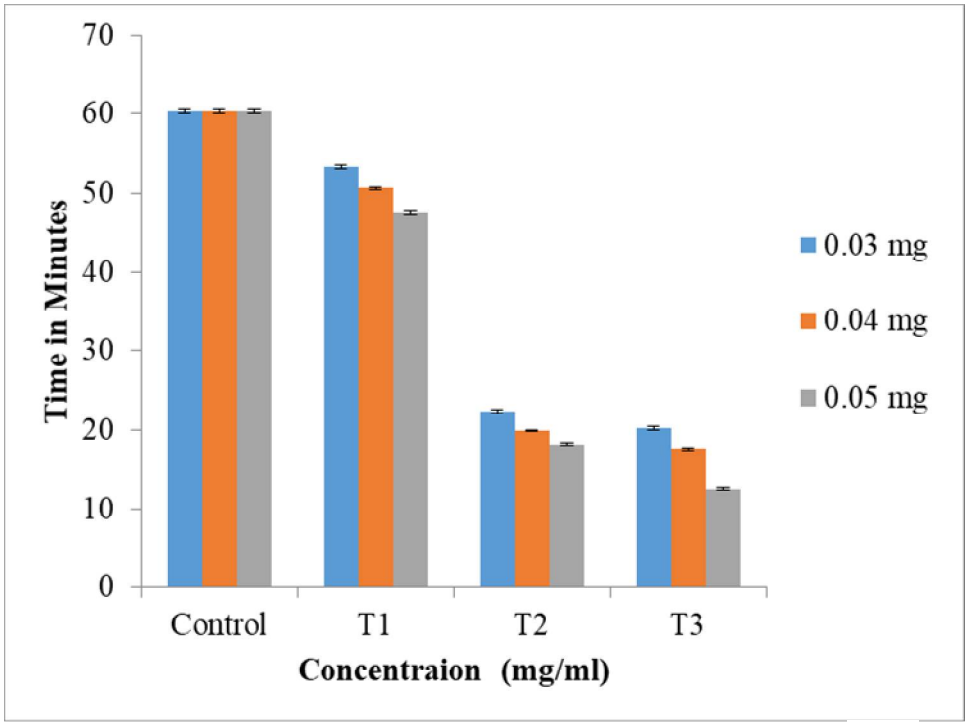

Fig 2. Mean (±SE) fecundity of *Drosophila melanogaster* on exposure to Nd_2^{+6} metal

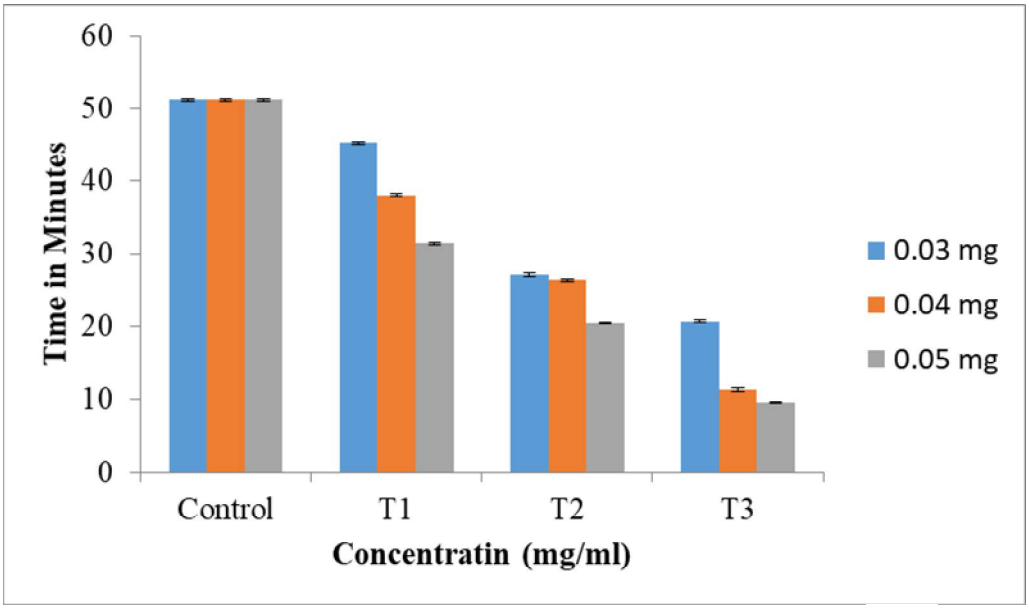

Fig 3. Mean (±SE) fertility of *Drosophila melanogaster* on exposure to Nd_2^{+6} metal

Table 1. Mean (±SE) of reproductive efficiency as life history traits in *Drosophila melanogaster* on magnetic exposure to Nd_2^{+6} metal

Traits	Copulation duration			Fecundity			Fertility		
Dose (mg) → / Trials ↓	0.03	0.04	0.05	0.03	0.04	0.05	0.03	0.04	0.05
C	21.7±0.73	21.7±0.73	21.7±0.73	60.3±0.26	60.3±0.26	60.3±0.26	51.2±0.21	51.2±0.21	51.2±0.21
T1	22.4±0.24	20.43±0.14	19.1±0.32**c**	53.3±0.21	38.2±0.20**c**	27.3±0.20**b**	45.2± 0.13	27.2±0.27**b**	20.7±0.16**a**
T2	19.5±0.25	17.46±0.13	15.33±0.18**c**	50.7±0.26	35.0±0.14**b**	23.8±0.15**a**	38.16±0.15**c**	26.15±0.15**b**	11.0± 0.19**a**
T3	16.2±0.13	14.6±0.12**c**	11.16±0.14**b**	47.5±0.25	29.6±0.13**b**	19.1±0.18**a**	31.5±0.14**c**	20.5±0.10**b**	9.5± 0.10**a**
ANOVA	df=3, 119 F=173.32 P<0.05	df=3, 119 F=364.57 P<0.05	df=3, 119 F=394.34 P<0.05	df=3, 119 F=488.39 P<0.05	df=3, 119 F=4041.33 P<0.05	df=3, 119 F=8552.29 P<0.05	df=3, 119 F=2722.43 P<0.05	df=3, 119 F=4701.82 P<0.05	df=3, 119 F=12377.42 P<0.05

C: Untreated ♀ × Untreated ♂; **T1**: Untreated ♀ × Treated ♂; **T2:** Treated ♀ × Untreated ♂: **T3**, Treated ♀ × Treated ♂
M±S.E. = Mean ± Standard Error; Duration: 04 days
DF= degree of freedom; F= F-test values; P<0.05 when compared with control

CONCLUSION

The current investigation reveals the *Drosophila* species as a useful tool to unravel the complex etiology of development. On exposure of graded doses of Nd_2^{+6} magnetic metal for the reciprocal crosses has revealed that any concentration continuous exposure can hinder the fitness of the organism whether it's a copulation duration, fecundity and fertility. Thereby Nd_2^{+6} metal exhibits the adverse effect at high doses when added to the diet of *Drosophila*. It shows the environmental Nd_2^{+6} metal pollution may affect the fruit fly diversity and it will be a loss of richness of such species due to poor performance due to environmental conditions. The further work focused on molecular mechanisms, evolutionary significance and other ethological conditions of *Drosophila* species to know the genetic compatibility during the variation in the exposure of Nd_2^{+6} magnetic rare earth elements.

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Nil

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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