Research Article

Effect of Lanthanum Chloride on Reproductive Efficiency of *Drosophila simulans*

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ABSTRACT

In present study, *Drosophila simulans* has been used as an experimental organism to know the efficacy of rare earth element (REE) metal, lanthanum chloride ($LaCl_3$) on the reproductive efficiency as life history traits viz., copulation duration, fecundity and fertility are assessed with a focused aim of exposure to lethal concentrations The REE metal was mixed in cream agar media of wheat in graded doses of ($LaCl_3$ 0.02mg, 0.03mg, 0.04mg). *D. simulans* species were cultured in laboratory under standard conditions in a defined media. Third instar stage of larvae's were used for the treatment by ingesting when they were actively crawling, boring and feeding voraciously. Results showed that on exposure of $LaCl_3$ graded doses of concentration to treated versus the control showed reduced fecundity and fertility significantly ($p<0.05$), while the copulation duration increased in the treated flies than that of the control with different crosses. The studied element exhibited significant effect on the reproductive parameters and it may harmful if exposed more than a 10mg concentration naturally and effect on maximum flies diversity.

Keywords: *Drosophila simulans*, Lanthanum chloride, Copulation duration, Fecundity, Fertility.

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INTRODUCTION

Rare earth element (REE) metals exist among the different compare to other minerals, such as carbonates, halides, oxides, phosphates, silicates, etc. The dominant Cerium, act as REE, is currently using in cars as catalytic converter, it will help to run and enabled them by increasing temperature, and also plays significant role by converting chemical reactions in the mechanism. It is also used in all the models of camera lenses and high-end telescopes. Compounds like lanthanum are recognised for the extensive applications such as a projectors, studios lights and cinema light projection as carbon lighting sources.[1]

Lanthanum is easily found in the 'rare earth' as metals, mainly monazite and bastnaesite (25% lanthanum and 38% lanthanum respectively). Various methods are using for separation of minerals, in that ion-exchange technique is very efficient and also solvent extraction method to obtain more yield are commonly used to isolate the 'rare earth' metal as key elements in the natural sources.[2] It is usually acquired by the reducing process of anhydrous fluoride along with calcium elements. It is very soft, flexible, spongy, whitish silver metal. It is naturally occurred earth surface, rapidly oxidizes with atmospheric air and also reacts easily with hydrogen to convert in to as hydroxide. In that, e 1:1 salt lanthanum phosphate ($LaPO_4$) has an extremely low solubility in water, similarly, lanthanum carbonate ($La_2(CO_3)_3$) also has a very low water solubility, 1.02×10^{-7}mol/L. Lanthanum is used in equipment such as colour televisions, fluorescent lamps, energy-saving lamps and glasses.[3]

La_2O_2 is used to make special optical glasses (infrared adsorbing glass, camera and telescope lenses). If added in small amounts it improves the malleability and resistance of steel. Lanthanum is used as the core material in carbon arc electrodes. Lanthanum salts

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are included in the zeolite catalysts used in petroleum refining because they stabilize the zeolite at high temperatures.[4] Lanthanum is used in large quantities in nickel metal hydride rechargeable batteries. The main problem with this element is its disposal. By the industrial wastes getting dumped into the water bodies mainly the petroleum producing industry. It produces severe damage to plants and animals and also causes nervous system disorder in humans.[5] Compounds containing lanthanum are extensively used extensively in carbon lighting applications, such as studio lighting and cinema projection. Used to make rechargeable lanthanum nickel metal hydride batteries, laptop, computers, high-end camera lenses, etc.[6] Metals may also be ingested involuntarily through food items. The amount that is absorbed from the digestive tract can vary widely, depending on the chemical form of the metal and the age and nutritional status of the individual. Once a metal is absorbed, it distributes in the tissues and organs. Excretion typically occurs primarily through the kidneys and digestive tract, but metals tend to persist in some storage sites like liver, bones and kidneys for years.[7] Hence there is a considerable interest in understanding the combined effects of heavy metals.

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 melanogaster* and *Drosophila simulans* are 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 simulans* to evaluate the effect of $LaCl_3$ toxicity on fitness of flies upon exposure to graded concentration.

MATERIALS 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°C (rearing temperature), 12 : 12 hr photoperiods and a relative humidity of 70%. The test flies were cultured in wheat cream agar media supplemented with graded concentrations of $LaCl_3$ (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; ($LaCl_3$ 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, three successive transfers were made into fresh food containing metal once in two days for the above said species of *Drosophila.*

Drosophila simulans 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 $LaCl_3$ 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 min. The pairs which do not mate within a stipulated time of 60 min were discarded.[15]

Fecundity and Fertility

Virgin females and unmated males of *Drosophila simulans* 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 $LaCl_3$ 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 $LaCl_3$ 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 $LaCl_3$ metal on copulation duration of *Drosophila simulans*

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 $LaCl_3$ 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. simulans* as shown in Table 1.

Effect of $LaCl_3$ metal on Fecundity of *Drosophila simulans*

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 lanthanum chloride 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 $LaCl_3$ metal on Fertility of *Drosophila simulans*

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 lanthanum chloride 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 simulans* as shown in Table 1.

DISCUSSION

Generally, studies on courtship behavior in experimental condition have concentrated on two role of transcription

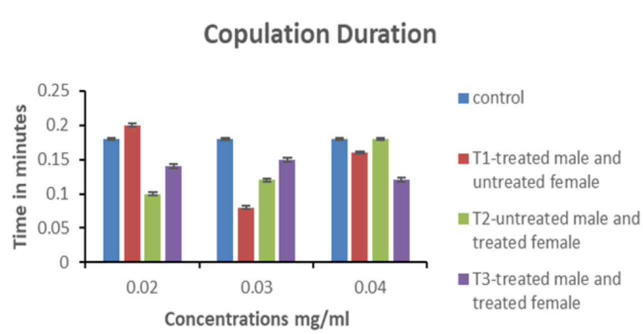

Figure 1: Mean (±SE) copulation duration of *Drosophila simulans* on exposure to $LaCl_3$ metal.

Table 1: Mean (±SE) of reproductive efficiency as life history traits in *Drosophila simulans* on exposure to $LaCl_3$ metal.

Traits	Copulation duration			Fecundity			Fertility		
Dose (mg) Trials	0.02	0.03	0.04	0.02	0.03	0.04	0.02	0.03	0.04
C	20.7±0.20	22.4±0.20	21.20±0.20	54.5±0.20	54.5±0.20	54.5±0.20	51.7±0.20	52.2±0.20	51.7±0.20
T1	15.50±0.18**c**	12.00±0.20**b**	6.90±0.14b**a**	45.40±0.20**a**	42.1±0.12**b**	36.0±0.20**c**	34.50± 0.14**c**	13.5±0.12**c**	24.50±0.15**c**
T2	12.30±0.20**b**	8.20±0.18**c**	3.15±0.20**a**	32.20±0.12**c**	29.0±0.20**a**	24.6±0.11**b**	23. 18±0.18**b**	19.7±0.14**b**	16.12±0.12**b**
T3	9.12±0.18**c**	4.10±0.10**b**	1.10±0.18**a**	21.21±0.18**c**	18.5±0.14**b**	13.2±0.14**a**	16.70±0.15**a**	9.0± 0.18**a**	3.12± 0.14**a**
ANOVA	df=3, 116 F=624.25 *P*<0.05	df=3, 116 F=2399.9 *P*<0.05	df=3, 116 F=2755.36 *P*<0.05	df=3, 116 F=4924.7 *P*<0.05	df=3, 116 F=16021.6 *P*<0.05	df=3, 116 F=7341.4 *P*<0.05	df=3, 116 F=7186.5 *P*<0.05	df=3, 116 F=12107.6 *P*<0.05	df=3, 116 F=15387.95 *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= degee of freedom; F= F-test values; *P*<0.05 when compared with control

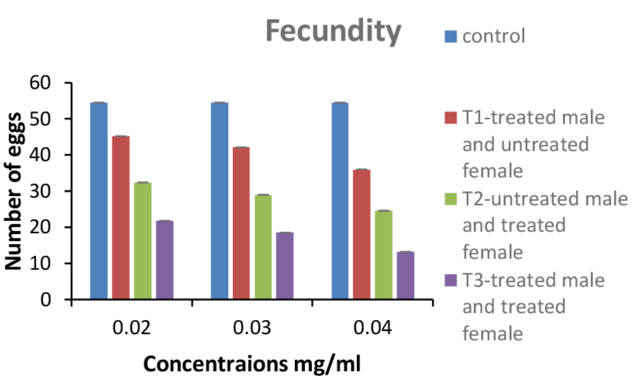

Figure 2: Mean (±SE) fecundity of *Drosophila simulans* on exposure to $LaCl_3$ metal.

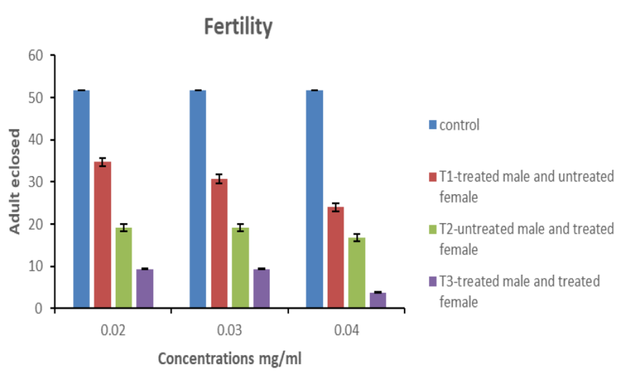

Figure 3: Mean (±SE) fertility of *Drosophila simulans* on exposure to $LaCl_3$ metal.

factors influence the Drosophila sex-determination in the hierarchy of fruitless (fru) and double sex (dsx). These factors exhibit to show sex-specific physiology and neural circuitry.[18] In the literature, fru and dsx factors regulate the courtship, expressed it differently from behavioral analyses in males and females showing mutations at the respective loci.[19] These genes regulate the sexual behavior and specify courtship behaviour as per the nutritional, temporal, and spatial patterns under the influence of nervous system, which establish to determine candidate cellular nature of the neuronal network.[18,20]

Rare earth element (REE) can lead to morphological changes of the liver. After REE enter the liver, they will interact with many proteins and other molecules in the cells. REE have effects on the activities of enzymes and interfere in physiological functions of the liver, via informational molecules.[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* consists of specific actions, which are accompanied by orientation movements made up of several signals, which are performed sequentially.[23] Factors influencing variation in duration of copulation are very complex and species-specific traits in *Drosophila*.[24]

Adult behaviour that involved 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 $LaCl_3$ 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 reduced compared to the results of pairs C, T1, T2. This could be due to differences in the dynamics of the interactions between the male and female when only one partner has been exposed to rare earth metal. When both partners have been exposed (T3), the general lack of interest in courtship and copulation are parallel to each other. In contrast, when only the males have been exposed, the males were not interested in the females. Furthermore, there were some instances where the females seemed to seek the male and the male stayed away from the female. When only the female was exposed to lanthanum chloride, the male vigorously attempted to court whereas females avoided contact with the male. A key gene known to be significantly involved in courtship behaviours is fruitless (fru), where almost every stage of the mating process has been shown to be disrupted by certain alleles of the locus.[26] The trivalent ion of a REE, $LaCl_3$ metal was studied for the effects on the growth, transformation, and gene expression of *Escherichia coli*. The results showed that La^{3+} at concentrations from 50 to 150 μg/mL stimulated both endogenic and ectogenic metabolism, but had few effects on gene expression. La^{3+} at lower concentrations (0.5-30 μg/mL) intensively inhibits *E. coli* absorbing external DNA, decreasing the transformation efficiency.[27] REE exposure in mononuclear cells from human peripheral blood (PBMNCs) increased the telomerase activity and the percentages of cells in the S-phase and the G2/M phase, but it had no effect on the apoptotic rate of PBMNCs. Under exposure to lower concentrations of RE, the telomerase activity of PBMNCs in the exposed group was higher than that of the control group, and there was no effect on the apoptotic rate of PBMNCs, but diploid DNA replication was promoted, and the percentages of G2/M- and S-phase cells increased.[28] In the present work, the values of the mean minimum and maximum concentrations of lanthanum chloride 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 simulans* flies.

CONCLUSION

The present findings accentuate the *Drosophila* species as a useful model system to unravel the complex etiology of development. On treating with graded doses of $LaCl_3$ metal for the reciprocal crosses has revealed that any dose concentration continuous exposure can hinder the fitness of the organism whether it's a copulation duration, fecundity and fertility. Thereby $LaCl_3$ metal exhibits the adverse effect at high doses when added to the diet of *Drosophila*. It shows the environmental $LaCl_3$ 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 focussed to explore on biochemical pathway and mechanisms of *Drosophila* species to know the gene expression during the variation in the exposure of $LaCl_3$ metal.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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