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Sodium fluoride adversely affects ovarian development and reproduction in *Drosophila melanogaster*

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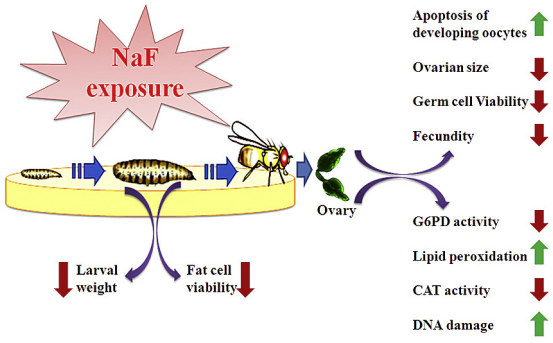
HIGHLIGHTS

- Chronic sub-lethal exposure to NaF (10–100 μg/mL) reduces fecundity in *Drosophila*.
- NaF exposure leads to altered adult body size and larval weight in fruit flies.
- NaF associated alterations in ovarian architecture and germ cell viability observed.
- NaF induces DNA fragmentation and changes in G6PD, CAT, LPO activities in ovary.
- NaF exposure at 10 and 20 μg/mL increases DNA damage in fat body cells.

GRAPHICAL ABSTRACT



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ABSTRACT

The study demonstrates the effects of chronic sub-lethal exposure of sodium fluoride (NaF) on reproductive structure and function of female *Drosophila melanogaster*. As a part of treatment, flies were maintained in food supplemented with sub-lethal concentrations of NaF (10–100 μg/mL). Fecundity, ovarian morphology, presence and profusion of viable cells from ovary and fat body were taken into consideration for evaluating changes in reproductive homeostasis. Wing length (a factor demonstrating body size and reproductive fitness) was also monitored after NaF exposure. Significant reduction in fecundity, alteration in ovarian morphology along with an increase in apoptosis was observed in treated females. Simultaneous decline in viable cell number and larval weight validates the result of MTT assay. Furthermore, altered ovarian Glucose-6-phosphate dehydrogenase and catalase activities together with increased rate of lipid peroxidation after 20 and 40 μg/mL NaF exposure confirmed the changes in reproduction related metabolism. Enhanced lipid peroxidation known for ROS generation might have induced genotoxicity which is confirmed through Comet assay. The enzyme activities were not dose dependent, rather manifested a bimodal response, which suggests a well-knit interaction among the players inducing stress and the ones that help establish physiological homeostasis.

1. Introduction

Any organism displays finest adaptability to the changing environment through sexual reproduction. Reproductive success

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is a measure of fitness of an organism which depends on several factors including process of copulation, fertilization, hatching of eggs and survivality of offsprings. Fecundity of females of a particular species determines the adaptive potential in the adverse environmental conditions. Likewise, the reproductive ability of insects are under the control of several extrinsic (temperature, humidity, light, diets) and intrinsic factors (body size, sex peptides, gene, age, metabolic processes) (Paul et al., 2001; Chapman et al., 2003; Regniere et al., 2012; Honek, 1993). Any variation in these factors might lead to alteration in several vital life processes like survival, mortality, fecundity, generation time etc. One such internal factor is body size which being a polygenic character is found to be influenced by changing environment (food and temperature) (Robertson, 1957) during insect development. In *Drosophila*, female body has a positive correlation with fecundity and mating success (Robertson, 1957). In addition to wing and thorax sizes, body weight is also an indicator for body size in *Drosophila* (Lefrance and Bundgaard, 2000).

Contrary to positive impact, types of negative relation between life history traits (increased reproductive effort with reduced survivorship) have also been demonstrated in insects (Mole and Zera, 1993). Forwarding ideas on trade-off, researches like Williams (1966); Reekie and Bazzaz (1987), have opined that, in conditions of limited resources, evolutionary alteration might result due to elevated resource allocation towards one trait and decreased allotment to another.

Anthropogenic as well as climatic variation leads to global changes that significantly affect organisms continuously exposed to such stresses (Harrison et al., 2006). Use of pesticides in our daily life causes environmental changes which has negative impact on the biology of non-target organisms. It has been reported that fluoride containing chemicals cryolite and sodium fluoride (NaF) causes alterations in compound eye morphology and developmental period in *D. melanogaster* (Podder et al., 2012; Dutta et al., 2014). Studies have demonstrated adverse effects of fluoride on fertility, fecundity and reproduction in several insects (Gerdes et al., 1971; Gong and Wu, 1991) including *Bombyx mori* where undesirable effect on reproductive cycle and fecundity is seen (Chen, 2003a; Chen, 2003b). Interestingly, lower birth rate in human has been found to be associated with exposure to drinking water with higher fluoride content (Freni, 1994).

Among the various fluorides, NaF used as the test chemical for the present study is commonly used for water fluoridation, water purification and manufacture of dental preparations like gel and varnishes etc (Green facts, 2002). Above processes/products might be responsible for elevated daily human consumption of fluoride (F^-). Hence fluoride exposure can be unique and erratic.

Thus the present study targets to explore the effects of chronic sub-lethal exposure of NaF on fecundity and reproductive physiology of *D. melanogaster,* which is considered as an alternative model organisms based on the recommendation from the European Centre for the Validation of Alternative Methods (Festing et al., 1998; Rand, 2010). Furthermore, several genes of *D. melanogaster* (*neplilysin, gustavus, vasa, ovo*) that are known to control the phenomenon of reproduction have their human homologs (Sitnik et al., 2014; Xing et al., 2006; Castrillon et al., 2000; Dai et al., 1998). In this context, any alteration in the studied parameters like fecundity, vitellogenesis, and activities of G6PD, Catalase, lipid peroxidase, cell viability and DNA degradation in *D. melanogaster* might serve as an indicator of NaF-induced reproductive toxicity in higher organisms including human. The effective dose might be higher for human because of their advanced reproductive physiology.

2. Materials and methods

2.1. Experimental organism

In the present study, *D. melanogaster,* Oregon R strain has been used as a model organism. *Drosophila* is maintained in standard *Drosophila* medium (SDM) containing sucrose, corn meal, agar, yeast, Nepagin, Propionic acid and distilled water at fixed ratio in laboratory within environmental test chamber at temperature range of (22–24)°C, (50–60)% humidity and 12 L:12D conditions. Flies used in this study have already been reared in the laboratory conditions approximately for more than 100 generations. Furthermore, parallel maintenance of insects of same age and from same batch but without chemical exposure as Control group help in comparison throughout the experiments.

2.2. Treatment schedule

Freshly hatched first instar larvae (~5–6 h of age) were exposed to different concentrations (10, 20, 40, 80, 100 μg/mL) of NaF (Merck Specialities Private Limited, Mumbai, India). The experimental larvae were maintained at a density of 100 larvae/plate in food supplemented with NaF until adulthood. 3rd instar larvae and adult female flies were used as experimental organisms for the parameters studied. The treatment concentrations (10, 20, 40, 80, 100 μg/mL) selected for this study were preferentially kept below the determined LC_{50} value (worked out in another study from our laboratory, communicated).

2.3. Analysis of fecundity

Keeping all parameters constant (temperature, age, density of larvae etc), fecundity was determined following the previously mentioned method (Memmi and Atli, 2011) with some modifications. Freshly eclosed 2 virgin male flies were allowed to mate with 1 virgin female (comprising one mating set) for 24 h, after which the males were removed and females placed in fresh food for egg laying. Since yeast is known to boost egg laying process, fresh food was supplied once in every 24 h. Eggs laid were counted up to 6th day post mating from ten mating sets for each treatment category in triplicate.

2.4. Body size (wing length and wing width)

Wing length and wing width are indicative of adult body size, hence adult wing size was measured following Orengo and Prevosti, 1999, with slight modifications. 4–5 days old gravid female flies from each treatment set were collected and preserved in 70% alcohol followed by phenol-alcohol (1:1) treatment. The dissected wings were and mounted using phenol-balsam mixture (Wirth and Marston, 1968).

Sum of the length of L1 (from the base of the 4th longitudinal vein to the posterior cross vein) and L2 (from the posterior cross vein to tip of the 4th longitudinal vein) was recorded. Furthermore, wing width (length between tip of the 5th vein and perpendicularly to the costal border) was also determined. All measurements were taken using oculometer, inserted in 5X ocular lens of compound microscope. Here 1 stage division (SD) is equal to 0.01 mm.

2.5. Ovary structure

The ovaries from 4 to 5 days old gravid female flies from each treatment category were dissected out in 1X phosphate buffered saline (PBS) and observed under simple binocular microscope.