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Amelioration of Fluoride Toxicity on the Reproductive Output of Drosophila melanogaster with L. Ascorbic Acid

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Abstract : *The study was conducted to assess the effect of sub-lethal concentration of Sodium Fluoride (NaF) on the reproductive output of Drosophila melanogaster and the possible ameliorative action of ascorbic acid on fluoride induced toxicity on the development of the fly. Results showed that there was significant fall in the number of 3rd instar larvae, pupae and ecloded flies in different concentrations of NaF (0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm). 1.0 ppm of NaF was lethal for the flies. When ascorbic acid (50 mM) was mixed in the medium, non-significant reduction in number of 3rd instar larvae and pupae was found in different concentrations of NaF. Further, flies survived in 1.0 ppm of NaF mixed with 50 mM ascorbic acid. It was concluded that L. Ascorbic acid helped to alleviate fluoride toxicity at least partially.*

Keywords: *Drosophila melanogaster, Sodium Fluoride, L. Ascorbic Acid.*

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Introduction:

Drosophila melanogaster has a life-span of about 30 days at 29°C (84°F) and the developmental period for *Drosophila melanogaster* varies with the temperature (Thompson and Woodruff, 1981). It takes 8-9 days to complete its life cycle. There are four different stages in the life cycle of *Drosophila melanogaster*, i.e. egg, larva, pupa and adult. The eggs hatch into first instar larvae which moult twice into second and third instar larvae. Third instar larvae pupate and finally metamorphose into adult flies.

Drosophila melanogaster is popularly used as a model to study toxic potential of any chemical (Jatav et al., 2011). Many studies have been conducted using *Drosophila melanogaster* in laboratory conditions to reveal well defined effects of various insecticides and pesticides on the life cycle, hatchability and emergence of the fly (Nazir et al., 2001; Nazir et al., 2003, Gupta et al., 2005; Das and Podder 2010).

Human activities as well as climatic variations have led to global changes which affect the organisms exposed (Harrison and Harrison, 2006). It has been reported that fluoride containing chemicals like cryolite and NaF can cause alterations in the compound eye morphology and

developmental stages in *Drosophila melanogaster (*Podder et al., 2012; Dutta et al., 2014).

Exposure to fluoride may affect the population of non-target organisms. Studies on toxic effects of NaF on the reproductive output of *Drosophila melanogaster* has not been done so far. Therefore, the present study was undertaken to document the effect of sub lethal dose of NaF on the reproductive output of *Drosophila melanogaster*.

In recent years, several investigations demonstrated that fluoride can induce oxidative stress and modulate intracellular redox homeostasis, lipid peroxidation and protein carbonyl content, as well as alter gene expression and cause apoptosis (Barbier et al., 2010).

Ascorbic acid is widely used as dietary supplement and increases the lifespan of animals (Ames, 1998). It is a strong anti-oxidant with an ability to neutralize free radicals. It also plays a protective role against oxidative stress, stimulates cell division and reproduction, and improves fertility (Yilmaz and Erkan 2015). Therefore, L. Ascorbic acid was used in this study to assess its possible role in amelioration of fluoride toxicity effects on the reproduction on the *Drosophila melanogaster.*

Materials and Methods :

Drosophila melanogaster were trapped from the garden of Patna Women's College. They were cultured in standard cornmeal medium.

Three sets of cultured bottles were kept in triplicates:

1) Control set: flies were cultured in normal cornmeal medium
2) NaF treated set : flies were cultured in cornmeal medium in which NaF was mixed in different concentration i.e. 0.2ppm, 0.4ppm, 0.6ppm, 0.8ppm, and 1.0ppm.
3) NaF + Ascorbic acid treated set: this set was similar to NaF treated set except that 50mM Ascorbic acid was added to each culture bottle.

Four adults (two males and two females) were added into each bottle and left undisturbed for nine days so that flies of next generation could emerge from the pupae. These flies were counted. This denoted the reproductive output of the initially added flies. Third instar larvae and pupae were also counted to find out which developmental stage was most affected.

The statistical analysis of the count data was performed using ANOVA. Because normality of data is a prerequisite for ANOVA, the count data were log transformed to ensure normal distribution. When the final F test in ANOVA indicated that there were significant differences between means of the count of the flies, Tukey test was performed to determine exactly which group differed most significantly from the control group.

Results and Discussion:

The present study found that 1.00 ppm of NaF was lethal for *Drosophila melanogaster*. Exposure of *Drosophila melanogaster* to sub-lethal dose of NaF did not affect the duration of life cycle. There was significant reduction in the number of 3rd instar larvae and pupae in different concentration of NaF as compared to control ($F=36.29$, $P<0.05$). (Tables 1 and 2). There was no change in number when third instar larvae changed into pupae suggesting sub-lethal concentration of NaF did not affect the growth phase. This may be due to the reason that ingestion of NaF with food during the larval life might have activated the drug-metabolizing enzymes. Drug-metabolizing enzymes have been reported in *Drosophila melanogaster* by Pai (1983).

Table 1. Number of 3rd instar larvae of Drosophila melanogaster at the end of 4th day of exposure to different concentrations of NaF

			NaF Concentration			
SET	**Initial**	**Control**	**0.2ppm**	**0.4ppm**	**0.6ppm**	**0.8ppm**
1	0.60 (4)	1.60 (40)	1.30 (20)	1.25 (18)	1 (10)	0.77 (6)
2	0.60 (4)	1.51 (33)	1.20 (16)	1.11 (13)	1 (10)	0.69 (5)
3	0.60 (4)	1.41 (26)	1.20 (16)	1.17 (15)	1.11 (13)	0.90 (8)
Mean±SE		1.5±0.05	1.2±0.03	1.17±0.04	1.03±0.03	0.8±0.04

Values are mean ±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses .

Table 2. Number of pupae of *Drosophila melanogaster* at the end of 5th day of exposure to different concentrations of NaF

			NaF Concentration			
SET	**Initial**	**Control**	**0.2ppm**	**0.4ppm**	**0.6ppm**	**0.8ppm**
1	0.60 (4)	1.60 (40)	1.30 (20)	1.25 (18)	1 (10)	0.77 (6)
2	0.60 (4)	1.51 (33)	1.20 (16)	1.11 (13)	1 (10)	0.69 (5)
3	0.60 (4)	1.41 (26)	1.20 (16)	1.17 (15)	1.11 (13)	0.90 (8)
Mean±SE		1.5±0.05	1.2±0.03	1.17±0.04	1.03±0.03	0.8±0.04

Values are mean ±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses.

There was significant reduction in the number of flies in the next generation in different concentrations of NaF as compared to control ($F = 42.33$, $P < 0.05$). Greatest reduction in reproductive output was seen in fly culture with 0.8 ppm of NaF in the cornmeal medium (Table 3). In the present study, exposure of *Drosophila melanogaster* to sub-lethal doses of NaF did not affect the duration of life cycle. However, Podder and Roy (2013) observed a distinct delay in emergence of flies in different concentrations of cryolite (sodium aluminium fluoride) as compared to control. Another study also demonstrated developmental delay in *Drosophila melanogaster* after chronic exposure to NaF (Dutta et al., 2014).

Further, fluoride induces oxidative stress in fluoride-intoxicated animals through generation of ROS and lipid peroxidation (MDA formation) (Chlubek, 2003). A significant depression was seen in the number of ecloded flies from the pupae. This may be because NaF could have caused the reduction of oxidative phosphorylation and ATP synthesis. The pupae require energy for morphogenesis and organogenesis. Reduction in the ability of ATP synthesis might have interfered with metamorphosis. NaF might also have interfered with hormones required for metamorphosis.

Table 3. Reproductive output of NaF treated flies after nine days of exposure to different concentration of NaF.

			NaF Concentration				
SET	**Initial**	**Control**	**0.2ppm**	**0.4ppm**	**0.6ppm**	**0.8ppm**	**1.0ppm**
1	0.60 (4)	1.6 (40)	1.25 (18)	1.17 (15)	1.04 (11)	0.69 (5)	All flies died
2	0.60 (4)	1.49 (31)	1.17 (15)	1.07 (12)	0.90 (08)	0.47 (3)	All flies died
3	0.60 (4)	1.54 (35)	1.20 (16)	1.14 (14)	1.04 (11)	0.77 (6)	All flies died
Mean±SE		1.55±0.03	1.2± 0.02	1.13±0.03	0.99±0.05	0.64±0.09	

Values are mean ±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses.

Table 4. Number of 3rd instar larvae of *Drosophila melanogaster* at the end of 4th day of exposure to different concentration of NaF + 50 mM of Ascorbic acid.

			NaF Concentration				
SET	**Initial**	**Control**	**0.2ppm**	**0.4ppm**	**0.6ppm**	**0.8ppm**	**1.0ppm**
1	0.60 (4)	1.34 (22)	1.39 (25)	1.32 (21)	1.20 (16)	1 (10)	0.84 (7)
2	0.60 (4)	1.50 (32)	1.38 (24)	1.25 (18)	1.11 (13)	0.84 (7)	0.69 (5)
3	0.60 (4)	1.53 (34)	1.32 (21)	1.30 (20)	1.17 (15)	1.95 (9)	0.69 (5)
Mean±SE		1.45±0.05	1.36±0.02	1.29±0.02	1.16±0.02	1.26±0.34	0.74±0.05

Values are mean ±SE.The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses.

Non-significant difference in the number of larvae and pupae was seen in different cornmeal media with ascorbic acid (F= 2.98, NS) (Tables 4 and 5). But, significant difference in the number of ecloded flies was seen in different cornmeal medium with ascorbic acid as well (F= 39.46, P<0.05) (Table 6).

Table 5. Number of pupae of Drosophila melanogaster at the end of 5th day of exposure to different concentration of NaF + 50mM of Ascorbic acid.

			NaF Concentration				
SET	***Initial***	***Control***	***0.2ppm***	***0.4ppm***	***0.6ppm***	***0.8ppm***	***1.0ppm***
1	0.60 (4)	1.34 (22)	1.39 (25)	1.32 (21)	1.20 (16)	1 (10)	0.84 (7)
2	0.60 (4)	1.50 (32)	1.38 (24)	1.25 (18)	1.11 (13)	0.84 (7)	0.69 (5)
3	0.60 (4)	1.53 (34)	1.32 (32)	1.30 (20)	1.17 (15)	1.95 (9)	0.69 (5)
Mean±SE		1.45±0.05	1.36±0.02	1.29±0.02	1.16±0.02	1.26±0.34	0.74±0.05

Values are mean ±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses.

Table 6. Reproductive output of NaF + 50 mM Ascorbic acid treated flies after nine days of exposure to different concentrations of NaF.

			NaF Concentration				
SET	***Initial***	***Control***	***0.2ppm***	***0.4ppm***	***0.6ppm***	***0.8ppm***	***1.0ppm***
1	0.60 (4)	1.34 (22)	1.39 (25)	1.30 (20)	1.17 (15)	0.9 (8)	0.69 (5)
2	0.60 (4)	1.43 (30)	1.34 (22)	1.23 (17)	1.04 (12)	0.69 (5)	0.3 (2)
3	0.60 (4)	1.27 (33)	1.30 (22)	1.27 (19)	1.11 (13)	0.84 (7)	0.47 (3)
Mean±SE		1.35±0.05	1.34±0.03	1.27±0.02	1.11±0.04	0.77±0.09	0.45±0.09

Value are mean ±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data data are given in parentheses.

In the present study it was found that 300 mM and 100 mM concentration of ascorbic acid were lethal for the flies and in 50 mM concentration of Ascorbic acid flies remained alive. 50mM ascorbic acid probably helped in reducing the toxicity of NaF as well. Massie et.al (1991) also investigated the effects of ascorbic acid on the life span of two *Drosophila* strains, *Oregon R and Swedish* C, at 10 mM concentration of ascorbic acid and found there was modest increase in the mean life span of *Oregon R* flies in comparison to the control. In contrast a concentration of 100 mM of ascorbic acid led to a significant decrease in the average life span of *Oregon R* flies.

Conclusion:

The present study concluded that NaF, which is a regularly used in toothpaste, insecticides and in water fluoridation program, can cause developmental alterations in non-target insects like *Drosophila melanogaster*, thereby suggesting its role in developmental toxicity. Ascorbic acid may be effective in reducing the effect of toxicity caused by NaF.

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