genes that causes disease in humans are also found in *Drosophila melanogaster* (Abolaji et al., 2015).

Studies have suggested that sub-lethal NaF exposure caused changes in reproduction and metabolism in female *Drosophila melanogaster* (Khatun et al., 2017). Studies also indicate a slight increase in climbing ability of fruit flies cultured in media with *Moringa oleifera* leaf extract (Iorjiim et al., 2020).

Medicinal plants possess therapeutic properties and serve as an alternative to synthetic medicines. One such highly valued plant is *Moringa oleifera* that is mostly cultivated in the tropics and subtropics (Moyo et al., 2011). It has an impressive range of medicinal uses with high nutritional value (high concentration of Vitamin A and C, tannins, phenols and flavonoids). Leaves of *Moringa oleifera* are the most widely used parts of the plant. The present study aims to test the toxicity of fluoride on *Drosophila melanogaster* and the possible protection by *Moringa oleifera* leaf extract on its survival and climbing ability.

**Materials and Methods :**

*D. melanogaster* was trapped and cultured in standard cornmeal media. Three sets of cultured bottles were kept in triplicates. Control set (with standard cornmeal media), Fluoride treated set (with different concentrations of Fluoride i.e., 1ppm, 0.75ppm and 0.5 ppm in cornmeal media), *Moringa* treated set (with 10% *Moringa* leaf extract added in the standard cornmeal media), Fluoride + *Moringa* treated set (with 10% *Moringa* leaf extract in 0.75ppm fluoride in the standard cornmeal media).

Life tables of flies of all the sets were prepared to determine the survival-mortality rate of *Drosophila melanogaster* in each media. Life table was prepared on the cohort of 45 flies. The numbers of dead flies were counted every day.

Climbing assay was performed according to Manjila and Hasan, (2018) in the glass cylinder of 50ml. Batch of 25 flies were used to perform climbing assay. Timer of 10 seconds was set and number of flies crossing 50ml border was counted. Experiment was repeated and average climbing ability of each batch of flies was calculated.

Lipid peroxidation assay was performed according to Ohkawa et al,(1979). 0.3 gm of flies were weighed and homogenised by adding 2ml of 0.5% Trichloroacetic acid (TCA) with the help of glass homogenizer. The homogenate was centrifuged at 5000rpm for 15 minutes at room temperature. 1ml of supernatant was transferred into clean and dry test tube. 2ml of freshly prepared 0.5% Thiobarbituric acid in 20% TCA was added into test tube containing centrifuged sample. Sample was incubated at 90°C for 30 minutes in water bath. Solution was cooled at room temperature. Absorbance was taken at 532 and 600nm. All the readings were taken in triplicate.

**Results and Discussion :**

In 1.0 ppm fluoride media, flies survived only for 4 days (Table 1). In 0.75ppm fluoride media, flies continued to survive and produced eggs and larvae for 6 days (Table 2). In 0.5ppm fluoride media, flies continued to survive and increase in number producing eggs, larvae as well as pupae for 11 days (Table 3). Hence 1.00ppm and 0.75ppm fluoride concentration were lethal for the flies while 0.5ppm was sub-lethal.

In the control media flies survived for 63 days (Table 4). In 0.75ppm fluoride media, flies survived for 28 days (Table 5) while flies in 0.5 ppm fluoride media survived for 35 days (Table 6). Flies in fluoride media (0.75ppm) + *Moringa* extract survived for 42 days (Table 7) showing better survival than fluoride media (both 0.5ppm and 0.75ppm) but less survival as compared to control media. In medium containing *Moringa,* flies

survived for 56 days (Table 8). *Moringa oleifera* is a rich source of ascorbic acid and flavonoids which have the antioxidant activity (Anwar et al, 2005). Fluoride toxicity can be reduced to a great extent through the use of *Moringa oleifera* leaves.

Climbing ability of *Drosophila melanogaster* in control media was maximum after 3 days and declined thereafter in 15 days (Table 9). Climbing ability in fluoride(0.75ppm) treated flies were very low as compared to control (Table 10) whereas flies in fluoride + *Moringa* extract showed better climbing as compared to fluoride treated flies (Table 11). In medium containing *Moringa,* climbing ability of flies were maximum as compared to flies cultured in other media (Table 12). Climbing activity of *Drosophila melanogaster* in different media is shown in Fig.1.

Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA (Gawel et al,2004). Hence, studies have shown that *Moringa* oleifera has antioxidant properties which reduces fluoride toxicity and oxidative stress (Gupta et al,2012). Lipid peroxidation assay revealed altered Malondialdehyde (MDA) production in the tissues of flies after exposure to sub-lethal concentration of fluoride (0.5ppm) was ~0.716mMol/g, while that of control flies was ~0.321mMol/g. MDA value in media with fluoride + *Moringa* extract was ~0.571mMol/g (Fig 2) and in media with *Moringa* extract was ~0.281mMol/g. Hence, MDA value observed was highest in fluoride treated flies and least in medium with *Moringa* extract (Fig.2).

The present study indicates that fluoride may be toxic to *Drosophila.* The toxic effects of fluoride cannot be ignored (Dhar and Bhatnagar 2009; Saverheber, 2013). The results of the present study indicate that aqueous *Moringa oleifera* leaf extract has the potential to provide protection against the toxic fluoride in *Drosophila melanogaster* and act as antioxidant by possibly scavenging oxygen free radical and other reactive oxygen intermediates and it suggests that *Moringa* may be of future therapeutic relevance particularly in the area where humans are chronically exposed to fluoride either occupationally or through food chain.

**Table 1. Culture of flies in 1ppm fluoride media**

| DAY | A | B | C |
|---|---|---|---|
| 1 | 6 flies | 6 flies | 6 flies |
| 2 | 5 flies+ eggs | 4 flies+ eggs | 4 flies |
| 3 | 4 flies+ no larvae | 2 flies+ no larvae | 3 flies+ eggs |
| 4 | 2 flies | – | 1 fly+ no larvae |
| 5 | – | – | – |

**Table 2. Culture of flies in 0.75ppm fluoride media**

| DAY | A | B | C |
|---|---|---|---|
| 1 | 6 flies | 6 flies | 6 flies |
| 2 | 6 flies | 5 flies | 5 flies + eggs |
| 3 | 5 flies | 5 flies + eggs | 5 flies + eggs |
| 4 | 4 flies | 4 flies + eggs | 3 flies, no larvae |
| 5 | 2 flies | 3 flies, no larvae | 2 flies, no larvae |
| 6 | – | 3 flies, no larva | 1 fly, no larvae |
| 7 | – | – | – |

**Table 3. Culture of flies in 0.5ppm fluoride media**

| DAY | A | B | C |
|---|---|---|---|
| 1 | 6 Flies | 6 flies | 6 flies |
| 2 | 6 flies + eggs | 6 flies + eggs | 6 flies + eggs |
| 3 | 6 flies + larvae | 6 flies + larvae | 5 flies + larvae |
| 4 | 6 flies + larvae | 6 flies + larvae | 5 flies + larvae |
| 5 | 6 flies + pupae | 5 flies + larvae | 5 flies + pupae |
| 6 | 5 flies + pupae | 5 flies + pupae | 4 flies + pupae |
| 7 | 5 flies + pupae | 4 flies + pupae | 4 flies + pupae |
| 8 | 4 flies + pupae | 3 flies + pupae | 3 flies + pupae |
| 9 | 3 flies + pupae | 3 flies + pupae | 12 flies |
| 10 | 12 flies | 14 flies | 12 flies |
| 11 | 12 flies | 14 flies | 15 flies |

**Table 4. Life table depicting survival of *Drosophila melanogaster* in control media**

| Sl. No. | Age interval x (In days) | No. surviving at beginning of the age interval (lx) | No. dying during the age interval x to x+1(dx) | Age specific mortality rate (qx) =dx/lx* 100 | Total number of days lived by flies x to x+1(Lx)= x+{x+1}/2 | Total number of days lived by the cohort (Tx) =lx1+ lx2..lxn | Average Life span (ex) = Tx/lx |
|---|---|---|---|---|---|---|---|
| 1 | 0-7 | 45 | 0 | 0 | 45 | 230 | 5.11 |
| 2 | 8-14 | 45 | 6 | 13.3 | 42 | 185 | 4.11 |
| 3 | 15-21 | 39 | 6 | 15.3 | 36 | 140 | 3.58 |
| 4 | 22-28 | 33 | 9 | 27.2 | 28.5 | 101 | 3.06 |
| 5 | 29-35 | 24 | 3 | 12.5 | 21 | 68 | 2.83 |
| 6 | 36-42 | 18 | 4 | 22.2 | 15 | 44 | 2.44 |
| 7 | 43-49 | 12 | 3 | 25 | 10.5 | 26 | 2.16 |
| 8 | 50-56 | 9 | 4 | 44.4 | 7 | 14 | 1.55 |
| 9 | 57-63 | 5 | 5 | 100 | 2.5 | 5 | 1.00 |
| 10 | 64-70 | 0 | - | - | - | - | - |

**Table 5. Life table depicting survival of *Drosophila melanogaster* in 0.75ppm fluoride media**

| Sl. No. | Age interval x (In days) | No. surviving at beginning of the age interval (lx) | No. dying during the age interval x to x+1(dx) | Age specific mortality rate (qx) =dx/lx* 100 | Total number of days lived by flies x to x+1(Lx)= x+{x+1}/2 | Total number of days lived by the cohort (Tx) =lx1+ lx2..lxn | Average Life span (ex) = Tx/lx |
|---|---|---|---|---|---|---|---|
| 1 | 0-7 | 45 | 8 | 17.7 | 36 | 87 | 1.93 |
| 2 | 8-14 | 27 | 15 | 55.5 | 19.5 | 42 | 1.55 |
| 3 | 15-21 | 12 | 9 | 75 | 7.5 | 15 | 1.25 |
| 4 | 22-28 | 3 | 3 | 100 | 1.5 | 3 | 1.00 |
| 5 | 29-35 | 0 | - | - | - | - | - |

**Table 6. Life table depicting survival of *Drosophila melanogaster* in 0.5ppm fluoride media**

| Sl. No. | Age interval x (In days) | No. surviving at beginning of the age interval (lx) | No. dying during the age interval x to x+1(dx) | Age specific mortality rate (qx) =dx/lx* 100 | Total number of days lived by flies x to x+1(Lx)= x+{x+1}/2 | Total number of days lived by the cohort (Tx) =lx1+ lx2..lxn | Average Life span (ex) = Tx/lx |
|---|---|---|---|---|---|---|---|
| 1 | 0-7 | 45 | 9 | 20 | 40.5 | 114 | 2.53 |
| 2 | 8-14 | 36 | 15 | 41.6 | 28.5 | 69 | 1.91 |
| 3 | 15-21 | 21 | 12 | 57.1 | 15 | 33 | 1.57 |
| 4 | 22-28 | 9 | 6 | 66.66 | 6 | 12 | 1.33 |
| 5 | 29-35 | 3 | 3 | 100 | 1.5 | 3 | 1.00 |
| 6 | 36-42 | - | - | - | - | - | - |

**Table 7. Life table depicting survival of *Drosophila melanogaster* in medium with fluoride + *Moringa* extract**

| Sl. No. | Age interval x (In days) | No. surviving at beginning of the age interval (lx) | No. dying during the age interval x to x+1(dx) | Age specific mortality rate (qx) =dx/lx* 100 | Total number of days lived by flies x to x+1(Lx)= x+{x+1}/2 | Total number of days lived by the cohort (Tx) =lx1+ lx2..lxn | Average Life span (ex) = Tx/lx |
|---|---|---|---|---|---|---|---|
| 1 | 0-7 | 45 | 12 | 26.6 | 28.5 | 133 | 2.95 |
| 2 | 8-14 | 33 | 9 | 27.2 | 21 | 88 | 2.66 |
| 3 | 15-21 | 24 | 6 | 25 | 15 | 55 | 2.29 |
| 4 | 22-28 | 18 | 9 | 50 | 13.5 | 31 | 1.72 |
| 5 | 29-35 | 9 | 5 | 55.5 | 6.5 | 13 | 1.44 |
| 6 | 36-42 | 4 | 4 | 100 | 2 | 4 | 1.00 |
| 7 | 43-50 | 0 | - | - | - | - | - |

**Table 8. Life table depicting survival of *Drosophila melanogaster* in medium containing *Moringa* extract**

| Sl. No. | Age interval x (In days) | No. surviving at beginning of the age interval (lx) | No. dying during the age interval x to x+1(dx) | Age specific mortality rate (qx) =dx/lx* 100 | Total number of days lived by flies x to x+1(Lx)= x+{x+1}/2 | Total number of days lived by the cohort (Tx) =lx1+ lx2..lxn | Average Life span (ex) = Tx/lx |
|---|---|---|---|---|---|---|---|
| 1 | 0-7 | 45 | 2 | 4.4 | 44 | 214 | 4.75 |
| 2 | 8-14 | 43 | 5 | 11.6 | 40.5 | 169 | 3.90 |
| 3 | 15-21 | 38 | 4 | 10.5 | 35 | 126 | 3.31 |
| 4 | 22-28 | 32 | 6 | 18.7 | 27.5 | 88 | 2.75 |
| 5 | 29-35 | 23 | 9 | 39.1 | 20 | 56 | 2.43 |
| 6 | 36-42 | 17 | 7 | 41.1 | 13.5 | 33 | 1.94 |
| 7 | 43-49 | 10 | 4 | 40 | 8 | 16 | 1.6 |
| 8 | 50-56 | 6 | 6 | 100 | 3 | 6 | 1 |
| 9 | 57-63 | 0 | - | - | - | - | - |

**Table 9. Climbing ability in *Drosophila melanogaster* in control medium**

| Sl. No. | A | B | C | Average no. of flies crossing 50mL border line of measuring cylinder |
|---|---|---|---|---|
| 1 | Day 1 after eclosion | 10s | 25 | 4.63 ± 0.16 |
| 2 | After 3 days | 10s | 25 | 11.3 ± 0.88 |
| 3 | After 7 days | 10s | 25 | 10.76 ± 0.50 |
| 4 | After 10 days | 10s | 25 | 6.66 ± 0.54 |
| 5 | After 15 days | 10s | 25 | 5.3 ± 0.41 |

**Table 10. Climbing ability of *Drosophila melanogaster* in Fluoride (0.75ppm) media**

| Sl. No. | Days | Time | Total Flies | Average no. of flies crossing 50mL border line of measuring cylinder |
|---|---|---|---|---|
| 1 | Day 1 after eclosion | 10s | 25 | 1.63± 0.27 |
| 2 | After 3 days | 10s | 25 | 5.53 ± 0.32 |
| 3 | After 7 days | 10s | 25 | 4.53 ± 0.64 |
| 4 | After 10 days | 10s | 25 | 2.63± 0.16 |
| 5 | After 15 days | 10s | 25 | 0.5± 0.08 |

**Table 11. Climbing ability of *Drosophila melanogaster* in *Moringa* + Fluoride (0.75ppm) media**

| Sl. No. | Days | Time | Total Flies | Average no. of flies crossing 50mL border line of measuring cylinder |
|---|---|---|---|---|
| 1 | Day 1 after eclosion | 10s | 25 | 9.83± 0.19 |
| 2 | After 3 days | 10s | 25 | 6.43 ± 0.24 |
| 3 | After 7 days | 10s | 25 | 7.53± 0.07 |
| 4 | After 10 days | 10s | 25 | 7.06± 0.19 |
| 5 | After 15 days | 10s | 25 | 6.8± 0.24 |

**Table 12. Climbing ability in *Drosophila melanogaster* in medium containing *Moringa***

| Sl. No. | Days | Time | Total Flies | Average no. of flies crossing 50mL border line of measuring cylinder |
|---|---|---|---|---|
| 1 | Day 1 after eclosion | 10s | 25 | 4.3± 0.13 |
| 2 | After 3 days | 10s | 25 | 10.06± 0.32 |
| 3 | After 7 days | 10s | 25 | 9.3± 0.12 |
| 4 | After 10 days | 10s | 25 | 10.93± 0.05 |
| 5 | After 15 days | 10s | 25 | 9.3± 0.14 |

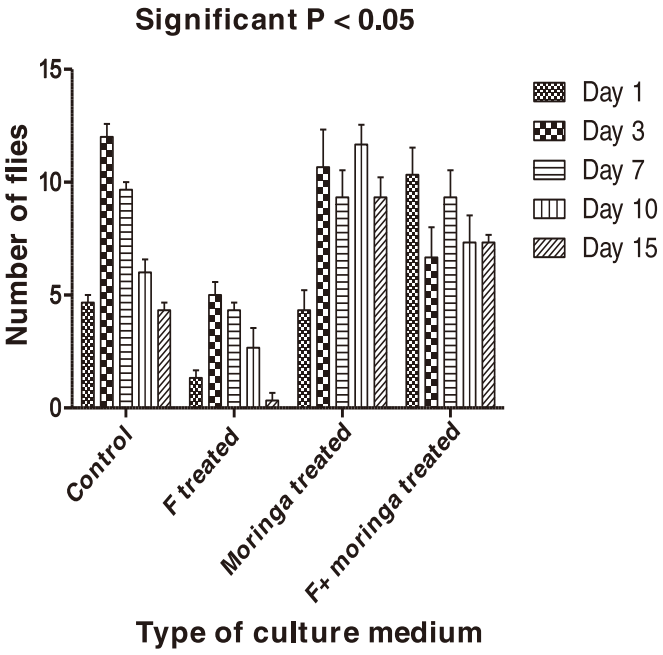


**Fig 1. Climbing activity of *Drosophila melanogaster* in different media**

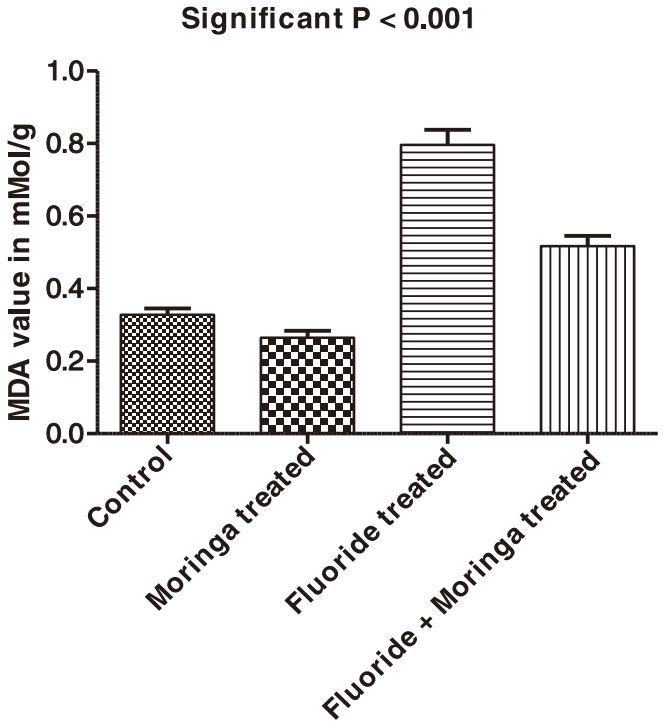


**Fig. 2. Graph showing MDA value of flies in different media**

**Conclusion :**

From the present work it can be concluded that exposure of *Drosophila melanogaster* to the sub – lethal concentration of fluoride induced toxic effects on the survival and climbing ability of fruit flies and caused oxidative stress. *Moringa oleifera* leaf extract has the ability to ameliorate the toxic effects of fluoride in fruit flies. Therefore, the study confirms the toxicity caused by fluoride at lethal and sub-lethal concentration in *D. melanogaster* and positive role of *Moringa oleifera* in curbing its effect.

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