nutraceutical properties. An important factor that accounts for the medicinal uses of *Moringa oleifera* is its very wide range of vital antioxidants, antibiotics and nutrients including vitamins and minerals (Faizal et al, 2014).This paper presents the possible amelioration of arsenic toxicity with the help of *Moringa* leaf extract mixed with food in *Drosophila melanogaster.*

Materials and Methods:

Fruitflies were trapped using plastic bottles with banana peel inside it. Three set of cultured bottles were kept in triplicates. Control set had normal cornmeal media. Arsenic trioxide treated set was prepared using different concentrations of arsenic i.e, 0.75 ppm, 0.5 ppm and 0.25 ppm in cornmeal media. *Moringa* containing media was prepared after adding 10% *Moringa* leaf extract in the standard cornmeal media. *Moringa* +arsenic media was prepared after adding 10% *Moringa* leaf extract in 0.25ppm and 0.5ppm of arsenic trioxide in the standard cornmeal media.

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

For preparing the life table, newly eclosed female flies were collected and raised in standard cornmeal media at 25˚C. 15 flies were placed per vial and were transferred to fresh vials every three days. The number of dead flies was counted every day. Survival of fruitflies in different media was assessed from the life table data. Age intervals of flies were considered in days.

For studying the reproductive output in different media, six adult flies, three males and three females were added into each vial. The vials were left undisturbed for 10 days so that flies of next generation could come out from the pupae. The emerged flies were counted. This showed the reproductive output of the initially added flies. Third instar larvae and pupae were also counted to observe which developmental stage was most affected.

The statistical analysis was performed using ANOVA.

Results and Discussion:

Flies cultured in 0.25 ppm arsenic medium continued to survive and reproduce (Table 1). Flies cultured in 0.5 ppm arsenic media survived for only six days. Some eggs but no larvae were found in the bottles with 0.5 ppm (Table 2). Flies cultured in 0.75 ppm arsenic media survived for only three days (Table 3). Flies cultured in 0.25 ppm arsenic media continued to survive and produce eggs and larvae. So, 0.25 ppm arsenic was considered as sub-lethal concentration for *Drosophila melanogaster* and 0.5 ppm and 0.75 ppm concentrations were considered as lethal.

50% of the flies cultured (out of 45) were dead between 28-35 days in the control media (Table 4), between 14-21 days in arsenic media (Table 5 and 6), between 28-35 days in media with *Moringa* extract (Table 7) and between 21-28 days in arsenic + *Moringa* containing media. Chronic exposure to arsenic and arsenic derivatives can have toxic effect (Miller et al, 2002).

Value of average life expectancy of flies cultured in control media was 1.85 by 35th day (Table 4) whereas life expectancy of flies cultured in arsenic media fell to 0 by the 28th day in 0.5 ppm

and by the 35th day in 0.25 ppm.(Table 5 and 6). Life expectancy of flies in media with arsenic+*Moringa* extract was observed to be 1.21 by the 28th day (Table 8), which was better than the arsenic treated flies with value of 0.5 by the 28th day (Table 5). Leaves of *Moringa oleifera* is useful in reducing the effects of arsenic-induced toxicity (Sheikh Afzal et al, 2014).

From the life table, survival of *Drosophila melanogaster* was seen to be more in the control medium (Table 4) as compared to medium containing 0.25 ppm arsenic (Table 5) where only one fly survived till 29 days and in medium containing 0.5 ppm (Table 6),where single fly survived till 21 days. Survival of flies in medium containing *Moringa* extract (Table 7) was comparable to control medium whereas survival of flies in arsenic+*Moringa* containing media (Table 8) was observed to be better than that in arsenic media.

Reproductive output of *Drosophila melanogaster* in the control medium after 10 days of observation found to be maximum. Flies treated with arsenic+*Moringa* had more reproductive output as compared to arsenic treated flies. This indicated that *Moringa* leaf extract helped to counteract the toxic effects of arsenic trioxide. (Table 9-14). Petursdottir et al (2015) also found that ingestion of arsenic trioxide by braconid wasps, resulted in a nonselective lowering of egg production.

Elevated oxidative stress is generally regarded as a pathological condition (Tsikas, 2017). Lipids are susceptible to oxidation and lipid peroxidation products are potential biomarkers for oxidative stress (Niki, 2008) Malondialdehyde (MDA) is the best investigated product of lipid peroxidation (Tsikas, 2017). Lipid peroxidation assay showed high Malonyldialdehyde (MDA) values in the arsenic treated flies as compared to control. (Fig. 1).

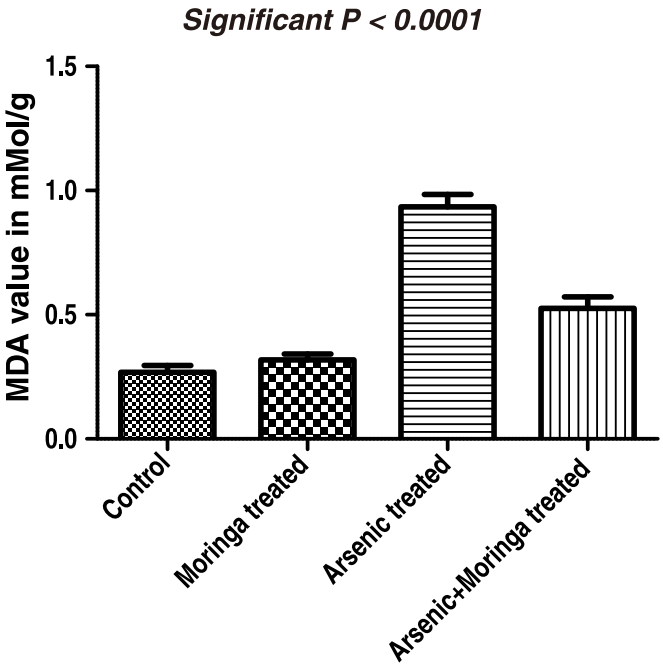

Fig.1. Graph showing MDA value of flies cultured in different media

Table 1. Culture of flies in 0.25 ppm arsenic trioxide

Date	A	B	C
20.11.20	7 flies	7 flies	7 flies
21.11.20	7 flies + eggs	7 flies+ eggs	7 flies+ eggs
22.11.20	7 flies + larvae	7 flies+ larvae	6 flies+ larvae
23.11.20	6 flies + larvae	7 flies+ larvae	6 flies+ larvae
24.11.20	6 flies + pupa	6 flies+ larvae	6 flies+ pupa
25.11.20	6 flies + pupa	5 flies+ pupa	6 flies+ pupa
26.11.20	5 flies + pupa	5 flies+ pupa	6 flies+ pupa
27.11.20	5 flies + pupa	5 flies+ pupa	6 flies+ pupa
28.11.20	~18 flies	4 flies+ pupa	5 flies+ pupa
29.11.20	~ 18 flies	~15 flies	~20 flies

Table 2. Culture of flies in 0.5 ppm arsenic trioxide

Date	A	B	C
20.11.20	7 flies	7 flies	7 flies
21.11.20	7 flies + eggs	7 flies + eggs	6 flies
22.11.20	7 flies + eggs	6 flies + no larvae	4 flies
23.11.20	5 flies + no larvae	6 flies + no larvae	4 flies
24.11.20	5 flies + no larvae	3 flies + no larvae	2 flies
25.11.20	4 flies + no larvae	-	2 flies
26.11.20	-	-	-

Table 3. Culture of flies in 0.75 ppm arsenic trioxide

Date	A	B	C
20.11.20	7 flies	7 flies	7 flies
21.11.20	3 flies	4 flies	2 flies
22.11.20	-	-	-

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

Sl. No.	Age interval x	No.surviving at the beginning of the age interval (lx)	No. dying during the age interval x to x+1(dx)	Age specific mortality rate(qx)	Average no. of days lived by flies x to x+1(Lx)	Total no. of days lived by the cohort (Tx)	Average life span (ex)
1	7 days	45	00	00	45	188.5	4.18
2	7-14 days	45	03	6.66	43.5	143.5	3.18
3	14-21 days	42	10	23.8	37	100	2.38
4	21-28 days	32	12	37.5	26	63	1.96
5	28-35 days	20	07	35	16.5	37	1.85
6	35-42 days	13	04	30.7	11	20.5	1.57
7	42-49 days	09	04	44.4	7	9.5	1.05
8	49-56 days	05	05	100	2.5	2.5	0.5
9	56-63days	00	-	-	-	-	-

Table 5. Life table depicting survival of *D. melanogaster* in 0.25 ppm arsenic trioxide.

Sl. No.	Age interval x	No. surviving at the beginning of the age interval (lx)	No. dying during the age interval x to x+1(dx)	Age specific mortality rate (qx)	Average no. of days lived by flies x to x+1(Lx)	Total no. of days lived by the cohort (Tx)	Average life span (ex)
1	7 days	45	10	22.2	40	81.5	1.81
2	7-14 days	35	16	45.7	27	41.5	1.18
3	14-21 days	19	14	73.68	12	14.5	0.76
4	21-28 days	5	5	100	2.5	2.5	0.5
5	28-35 days	00	-	-	-	-	-

Table 6. Life table depicting survival of *D. melanogaster* in 0.5 ppm arsenic trioxide.

Sl. No.	Age interval x	No. surviving at the beginning of the age interval (lx)	No. dying during the age interval x to x+1(dx)	Age specific mortality rate (qx)	Average no. of days lived by flies x to x+1(Lx)	Total no. of days lived by the cohort (Tx)	Average life span (ex)
1	7 days	45	11	24.4	39.5	74.5	1.83
2	7-14 days	34	16	36.36	26	35	1.34
3	14-21 days	18	18	100	9	9	1
4	21-28 days	0	-	-	-	-	-

Table 7. Life table depicting survival of *D. melanogaster* in medium with *Moringa* extract

Sl. No.	Age interval x	No. surviving at the beginning of the age interval (lx)	No. dying during the age interval x to x+1(dx)	Age specific mortality rate (qx)	Average no. of days lived by flies x to x+1(Lx)	Total no. of days lived by the cohort (Tx)	Average life span (ex)
1	7 days	45	1	2.22	44.5	188.5	4.18
2	7-14 days	44	8	18.1	40	144	3.27
3	14-21 days	36	2	5.55	35	104	2.88
4	21-28 days	34	12	35.2	28	69	2.02
5	28-35 days	22	7	31.8	18.5	41	1.86
6	35-42 days	15	5	33.3	12.5	22.5	1.5
7	42-49 days	10	5	50	7.5	10	1.0
8	49-56 days	05	5	100	2.5	2.5	0.5
9	56-63 days	00	-	-	-	-	-

Table 8. Life table depicting survival of *D. melanogaster* in medium with arsenic and *Moringa*.

Sl. No.	Age interval x	No. surviving at the beginning of the age interval (lx)	No. dying during the age interval x to x+1(dx)	Age specific mortality rate (qx)	Average no. of days lived by flies x to x+1(Lx)	Total no. of days lived by the cohort (Tx)	Average life span (ex)
1	7 days	45	7	15.5	41.5	109.5	2.43
2	7-14 days	38	13	34.21	31.5	68	1.78
3	14-21 days	25	11	44	19.5	36.5	1.46
4	21-28 days	14	09	64.28	10.5	17	1.21
5	28-35 days	07	04	57.14	5	6.5	0.92
6	35-42 days	03	03	100	1.5	1.5	0.5
7	42-49 days	00	-	-	-	-	-

Table 9. Number of 3rd instar larva of *Drosophila melanogaster* at the 5th day of exposure to different concentration of As_20_3

SET	INITIAL	CONTROL	0.25ppm	0.5ppm
1.	0.77(6)	1.77(60)	1.47(30)	-
2.	0.77(6)	1.73(54)	1.41(26)	-
3.	0.77(6)	1.68(48)	1.41(26)	-
MEAN±SE		1.72±0.02	1.43±0.016	-

Table 10. Number of pupae of *Drosophila melanogaster* at the 6th day of exposure to different concentration of As_20_3

SET	INITIAL	CONTROL	0.25ppm	0.5ppm
1.	0.77(6)	1.77(60)	1.47(30)	-
2.	0.77(6)	1.73(54)	1.41(26)	-
3.	0.77(6)	1.68(48)	1.41(26)	-
MEAN± SE		1.72±0.02	1.43±0.016	-

Table 11. Number of *Drosophila melanogaster* at the 10th day of exposure to different concentration of As_20_3

SET	INITIAL	CONTROL	0.25ppm	0.5ppm
1.	0.77(6)	1.77(60)	1.36(23)	-
2.	0.77(6)	1.69(50)	1.32(21)	-
3.	0.77(6)	1.65(45)	1.27(19)	-
MEAN±SE		1.70±0.028	1.31±0.021	-

Table 12. Number of 3rd instar larva of *Drosophila melanogaster* at the 5th day of exposure to different concentration of As_20_3 + *Moringa* leaf extract.

SET	INITIAL	CONTROL	0.25ppm	0.5ppm
1.	0.77(6)	1.76(58)	1.51(33)	1.47(30)
2.	0.77(6)	1.73(54)	1.44(28)	1.38(24)
3.	0.77(6)	1.70(51)	1.44(28)	1.34(22)
MEAN±SE		1.73±0.13	1.46±0.018	1.39±0.31

Table 13. Number of pupae of *Drosophila melanogaster* at the 6th day of exposure to different concentration of As_20_3 + *Moringa* leaf extract.

SET	INITIAL	CONTROL	0.25ppm	0.5ppm
1.	0.77(6)	1.76(58)	1.51(33)	1.47(30)
2.	0.77(6)	1.73(54)	1.44(28)	1.38(24)
3.	0.77(6)	1.70(51)	1.44(28)	1.34(22)
MEAN±SE		1.73±0.13	1.46±0.018	1.39±0.31

Table 14. Number of *Drosophila melanogaster* at the 10th day of exposure to different concentration of As_20_3 + *Moringa* leaf extract.

SET	INITIAL	CONTROL	0.25ppm	0.5ppm
1.	0.77(6)	1.76(58)	1.5(32)	1.47(30)
2.	0.77(6)	1.72(53)	1.44(28)	1.38(24)
3.	0.77(6)	1.69(50)	1.41(26)	1.44(20)
MEAN±SE		1.73±0.016	1.46±0.02	1.39±0.02

Conclusion :

It can be concluded from the present work that the exposure of *Drosophila melanogaster* to the sub lethal concentration of arsenic trioxide showed toxic effects of arsenic trioxide on the reproductive rate as well as survival of the fly. The sublethal concentration of arsenic caused oxidative stress in *Drosophila melanogaster.* Aqueous *Moringa* oleifera leaf extract has the capability to counteract the toxic affect of arsenic trioxide on the flies and it can be useful as an antioxidant supplement. Hence, the study confirms the toxicity caused by arsenic trioxide at lethal and sublethal concentration in *Drosophila melanogaster* and *Moringa oleifera* as a possible curative agent.

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