AMELIORATIVE EFFECT OF CURCUMIN AND SOYA EXTRACT AGAINST ARSENIC TOXICITY IN *Drosophila melanogaster*

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ABSTRACT

Arsenic (As), a naturally occurring element may induce oxidative stress and is a potent carcinogen. The present study aimed to find the lethal and sub-lethal concentration of arsenic trioxide and explore its effect on survival of fruit fly *Drosophila melanogaster*. Amelioration using polyphenols (curcumin and soya extract) have been introduced as a successful strategy to overcome this problem. The study showed significant decrease in lifespan of flies treated with arsenic trioxide. 50% mortality was seen in media with 0.5 mM arsenic trioxide and 100% mortality at 0.75 mM. Increased lifespan was observed in medium containing arsenic trioxide (0.55mM) with curcumin (*Curcuma longa*) and soya extract (*Glycine max*) respectively. Best survival rate was found in 0.55mM arsenic medium with 1 mM Curcumin and 1.5 mM Soya extract respectively. Lipid peroxidation assay showed significant increase in malondialdehyde (MDA) values in the flies treated with arsenic trioxide as compared to control. The MDA values decreased significantly in flies treated with arsenic mixed with curcumin and soya extract respectively. The results indicate that exposure to sub lethal concentration of arsenic trioxide may cause oxidative stress and affect the lifespan of *D. melanogaster*. Curcumin and soya extract may help in reducing the impact of oxidative stress and toxicity caused by arsenic trioxide.

Keywords: Arsenic trioxide, *Curcuma longa*, *Glycine max*, Lifespan, Oxidative stress

INTRODUCTION

Arsenic is a metalloid occurring naturally in soil, air, and water (**Huang et al., 2004; Duker et al., 2005**). It exists in both organic and inorganic forms in the environment where inorganic arsenicals are more toxic in nature.

Although arsenic is an important component of few homeopathic medicines, organism's normal body functioning is vulnerable to its detrimental impacts when introduced to its high concentrations. Arsenic poisoning has thrown a new challenge to the survival of mankind. Its primary source in soil is parent rock (**Smedly and Kinniburgh, 2002**), and volcanoes in natural water (**Nriagu and Pacyna, 1988; Smedly and Kinniburgh, 2002**). Thus, it gets bioaccumulated in organisms. Arsenic is a potent carcinogen and its chronic exposure uncovers greater risk of cancer of the skin, lung and liver in human beings (**Banerjee et al., 2011**). It produces developmental toxicity, including malformation, death, and growth retardation. It also interferes with the plant metabolism and results in the interruption of photosynthesis, the sole phenomenon ensuring sustainability of life on earth.

Drosophila melanogaster being a non-target organism is widely used as an efficient biological model for various studies due to existing genetic homology with mammals and in particular humans (**Mackay and Anholt, 2006**). It is used for evaluation and assessment of various parameters in the field of genetics, neurology, biotechnology and toxicology. *Drosophila* can be utilized as a model as their short generation time enables to determine the effect of toxicants at different biological stages including larval, pupal and adult forms (**Peterson and Long, 2018**). Due to presence of highly conserved genes, it helps to understand human condition under stress of toxicants because of presence of same pathways controlling development and stress response (**Mackay and Anholt, 2006**).

Since time immemorial plant and plant products are used for treatment of diseases because of their effectiveness, low incidences of serious adverse effects and low cost (**Bhattacharya and Haldar, 2013**). Arsenic possesses the capacity to liberate the free radicals in our body by hindering the anti-oxidant mechanisms of our body. It also interferes with female reproductive cycle by inhibiting follicle maturation in ovaries **(Dávila-Esqueda et al., 2012)**. This complication can be ameliorated by treatment with an active anti-oxidant. Polyphenols such as 'curcumin' (*Curcuma longa*) and 'soya extract' (*Glycine max*) have been widely screened for their pharmacological effects as they inhibit carcinogenesis and have excellent chemo preventive properties. Curcumin, a dietary polyphenol has been reported to have anti- inflammatory and antioxidant properties (**Suzuki et al., 2009**). It augments metabolic syndrome, arthritis, anxiety, and hyperlipidemia. Soy is known to reduce the chances of chronic heart disease as well as breast and prostate cancer. It also helps to keep the bone healthy and provides relief of menopausal symptoms (**Messina, 2016**). This paper explores the ameliorative effect of curcumin and soya extract on arsenic toxicity in fruit fly, *D. melanogaster*.

MATERIALS AND METHOD

Native D. melanogaster was caught and cultured in the laboratory in standard cornmeal medium at 25 °C. Single gravid fly was allowed to lay eggs in the media. The eggs hatched to produce fly of same age group. The process was repeated to create single line stock culture.

For determining the lethal and sub lethal concentration of arsenic for D. melanogaster, arsenic trioxide solution at different concentrations (0.25 mM, 0.5 mM, 0.75 mM and 1 mM) was mixed with the standard corn meal media of the flies. Three bottles of each concentration were prepared. Ten flies from the stock were transferred into each set and monitored.

Preparation of stock solution of curcumin: 100 ml stock solution of curcumin was prepared by dissolving 36.83 g of pure curcumin powder, in 100ml Dimethylsulfoxide (DMSO).

Preparation of media having curcumin and arsenic trioxide: For preparing 100 ml of curcumin + arsenic trioxide cornmeal media, the working concentration of arsenic trioxide was mixed with different concentrations of curcumin (0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM and 1.2 mM) respectively.

Preparation of stock solution of soya extract: Soya bean seeds were collected and dried in hot air oven for 12 hours and was then crushed into coarse granules by using mortar and pestle following Woodruff et al (1938). The powdered form was soaked in methanol for seven days. The aqueous extract was then prepared by using Rotatory vacuum Evaporator. The extract obtained was then dissolved in 100 ml Dimethylsulfoxide.

Preparation of media having soya extract and arsenic trioxide: Different concentrations of soya extract + arsenic trioxide media (0.2 mM, 0.4 mM, 0.6 mM, 0.8

mM, 1.0 mM, 1.2 mM, 1.4mM, 1.5 mM and 1.6mM) were prepared in the similar fashion as that of the curcumin . Three bottles of each concentration were prepared.

For studying the effect of different media mentioned above on the lifespan of native fly, newly emerged flies were collected from the stock and raised in the respective media at 25 °C. Ten flies were placed into each bottle. The numbers of flies alive were counted every day.

Lipid peroxidation assay was performed on third generation flies exposed to different medium mentioned above following **Ohkawa et al (1979)**. In a glass homogenizer 0.3 g of the flies was taken and homogenized by adding 1 ml of 0.1% trichloroacetic acid (TCA). The homogenate was then centrifuged at room temperature for 15 min at 5000 rpm. To a clean and a dry test tube one milliliter of the supernatant was transferred. To it 2 ml of freshly prepared 0.5% thiobarbituric acid (TBA) in 20% TCA was added. This sample was then heated in a water bath for 30 min at 90 °C and was subsequently cooled at room temperature. Absorbance of sample was measured by dual beam spectrophotometer at wavelength 532 and 600 nm.

MDA level was calculated by following formula:

$$MDA = (OD\ 532 – OD\ 600\ X\ 100/1.56)\ X\ TV\ /\ [dw\ X\ 1000]$$

Where,

OD = optical density

TV = sample total volume dw = sample dry weight

The statistical analysis of the count data was performed using ANOVA and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Flies cultured in normal corn meal medium (control) survived for more than four weeks (Table 1). Acute toxicity was seen in 4 days and chronic toxicity in 7 days similar to the observations by **Goldstein and Babich, (1989)**. Survival of flies decreased significantly with the increasing concentration of arsenic in the media [$F_{(4, 65)} = 13.58$, $P < 0.01$] (Fig 1). 100% mortality was seen at 0.75 mM arsenic trioxide and thus it was considered as lethal concentration for flies. 50% mortality was seen at 0.5mM arsenic trioxide. Thus, 0.5 mM was observed as LC_{50} dose (Table 2). On the basis of the result observed, 0.55mM was taken as the working concentration for the flies.

The present study revealed that survival of flies in arsenic treated media was less as compared to the control. This significant reduction in the lifespan of flies is similar to the findings of **Goldstein and Babich (1989)**.

The problem caused by arsenic can be addressed through Ayurveda which focuses on healthy herbs such as curcumin (*Curcuma longa*), an active ingredient of turmeric and soya (*Glycine max*). Survival of flies cultured in curcumin containing medium [$F_{(5, 36)} = 0.02$; $P = 0.9996$] and soya extract containing medium [$F_{(8, 54)} = 0.03$; $P>0.9999$] (Fig 2) was comparable to control medium (Table 1). No significant difference was found in the three replicate bottles of both experimental setup. For determining the effects of genetic and non-genetic factors involved in ageing, lifespan measurement is one of the basic method (**Finch and Ruvkun, 2001)**.

Environmental factor such as diet has huge impact on lifespan of *Drosophila* and many other species **(Piper et al., 2011)**.

In the present study, significant ameliorative potential of curcumin [$F_{(6, 30)} = 34.56$; $P<0.0001$] and soya extract [$F_{(6, 48)} = 82.64$; $P<0.0001$] was observed (Fig 3).

Most of the therapeutic benefits of curcumin supplementations are due to its anti-inflammatory and anti-oxidant properties **(Aggarwal and Harikumar, 2009; Gupta et al., 2013)**. It has been shown to improve systemic markers of oxidative stress **(Sahebkar et al., 2013)**. On the other hand, consumption of soy protein or associated isoflavones has beneficial impacts on the risk factors for cardiovascular disease by lowering liver or blood triglyceride (**Anderson et al., 1995; Anthony et al., 1998; Lin et al., 2004; Moriyama et al., 2004**).

Since, long term exposure to arsenic causes skin lesion, cancer, cardiovascular disease and leads to increase in ROS in our body, therefore in this study curcumin and soya extract were found to be effective against the toxic effects of arsenic in fruit fly. Best survival rate was found in 0.55 mM arsenic containing media with 1 mM curcumin (Table 3; Fig 4) and 1.5 mM soya extract (Table 3; Fig 4) respectively.

Significant difference [Row Factor: $F_{(1, 6)} = 28.70$; Column factor: $F_{(1.59, 9.55)} = 13.71$; $P < 0.01$] were found in experimental setup of curcumin and curcumin + arsenic trioxide.

Significant difference [Row Factor: $F_{(1, 6)} = 30.16$; Column factor: $F_{(1.50, 9.02)} = 19.05$; $P < 0.01$] were found in experimental setup of soya extract and soya extract +arsenic trioxide.

Lipid peroxidation (LPO) assay revealed change in level of MDA produced in the tissues of flies exposed to different media [$F_{(5, 12)} = 18449$; $P < 0.0001$] (Fig 5). Level of MDA was highest in arsenic treated flies. Lipid peroxidation (LPO) assay, a marker to measure oxidative stress, revealed altered Malonyl dialdehyde (MDA) production in the tissues of flies after exposure to arsenic trioxide. Arsenic causes oxidative stress by forming super oxide radical ion, hydrogen peroxide, hydroxyl radical, hydroperoxyl radical, peroxyl radical and singlet oxygen. It alters signal transduction via ROS alteration or reversible oxidation of -SH group in proteins, which leads to activation or

inhibition of transcription factors and regulates gene transcription **(Platanias, 2009)**.

In the present study, decrease in the level of MDA was observed in flies treated with curcumin and soya extract respectively. Thus, the result indicates that aqueous extract of curcumin and soya seeds may act as antioxidants to provide protection against the arsenic toxicity in fruit fly, *Drosophila melanogaster*.

CONCLUSION

From the present study it can be concluded that exposure of *D. melanogaster* to arsenic trioxide caused oxidative stress which was reflected in their lifespan as substantial decrease in survival was observed. It was found that curcumin and soya extract have ameliorative effect against arsenic toxicity. This suggests that curcumin and soya extract can be useful as a dietary antioxidant supplement, scavenging oxygen free radical and other reactive oxygen intermediates. They have promising antioxidant potential to combat arsenic induced toxicity.

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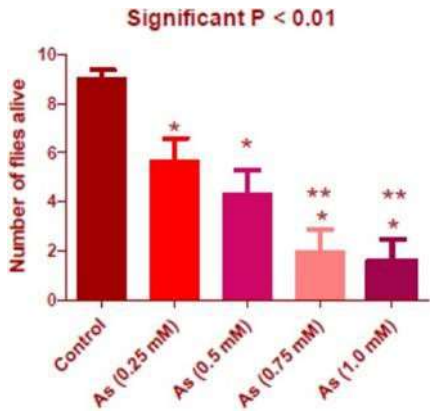
TABLE, GRAPHS AND CHARTS

Table 1. Survival of flies in control medium

WEEK	SET 'A'	SET 'B'	SET 'C'	MEAN ± SE
WEEK 1	10	10	10	10 ± 0
WEEK 2	10	8	10	9.33 ± 0.54
WEEK 3	9	8	8	8.33 ± 0.27
WEEK 4	8	6	6	6.67 ± 0.54
WEEK 5	7	5	6	6.00 ± 0.47
WEEK 6	5	5	3	4.33 ± 0.54
WEEK 7	3	3	2	2.67 ± 0.27
WEEK 8	ALL DEAD	ALL DEAD	ALL DEAD	~

Table 2. Survival of flies in Arsenic medium

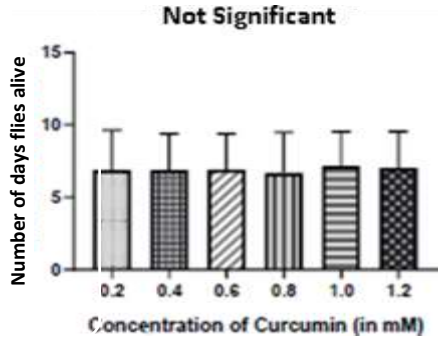
	ARSENIC 0.5 milimoles				ARSENIC 0.75 milimoles			
DATE	SET 'A'	SET 'B'	SET 'C'	MEAN ± SE	SET 'A'	SET 'B'	SET 'C'	MEAN ± SE
30.09.2021	10	10	10	10±0	10	10	10	10±0
01.10.2021	10	10	9	9.67±0.27	9	8	6	7.67±0.72
02.10.2021	8	9	8	8.33±0.27	6	5	5	5.33±0.27
03.10.2021	**8**	**7**	**7**	**7.33 ±0.27**	**3**	**2**	**5**	**3.33±0.72**
04.10.2021	6	5	6	5.67±0.27	1	ALL DEAD	2	1±0.33
05.10.2021	6	5	6	5.67±0.27	ALL DEAD	ALL DEAD	1	0.33±0.27
06.10.2021	**6**	**5**	**4**	**5±0.47**	ALL DEAD	ALL DEAD	ALL DEAD	~
07.10.2021	5	4	4	4.33 ± 0.27	ALL DEAD	ALL DEAD	ALL DEAD	~
08.10.2021	3	3	3	3 ± 0	ALL DEAD	ALL DEAD	ALL DEAD	~
09.10.2021	1	2	1	1.33 ± 0.27	ALL DEAD	ALL DEAD	ALL DEAD	~
10.10.2021	ALL DEAD	1	ALL DEAD	0.33 ± 0.27	ALL DEAD	ALL DEAD	ALL DEAD	~
11.10.2021	ALL DEAD	ALL DEAD	ALL DEAD	~	ALL DEAD	ALL DEAD	ALL DEAD	~



*Statistically significant w.r.t. control
* Statistically significant w.r.t. 0.25mM As Concentration

Fig 1. Survival of flies in control and media containing different concentrations of Arsenic trioxide

a)



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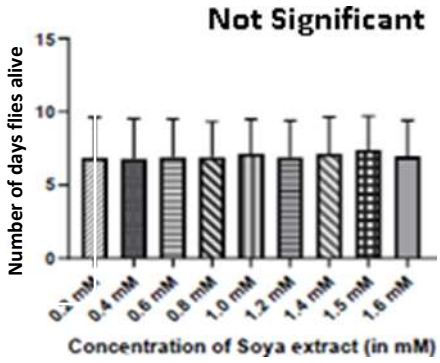
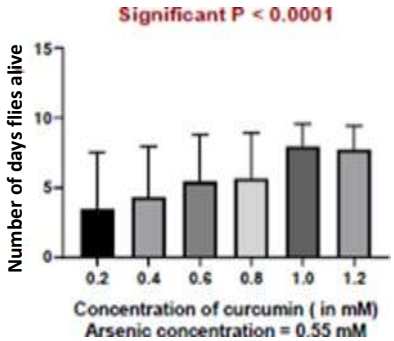


Fig 2. Number of days flies survived at different concentration in (a) Curcumin containing media and (b) Soya Extract containing media

a)



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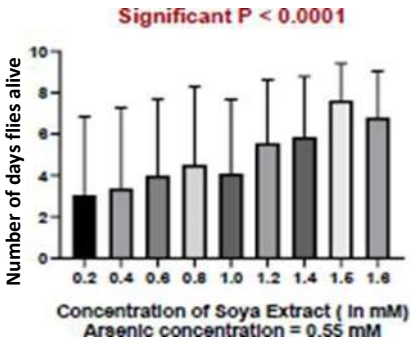

Fig 3. Number of days flies survived in 0.55mM arsenic trioxide containing media at different concentration of (a) curcumin and (b) soya extract

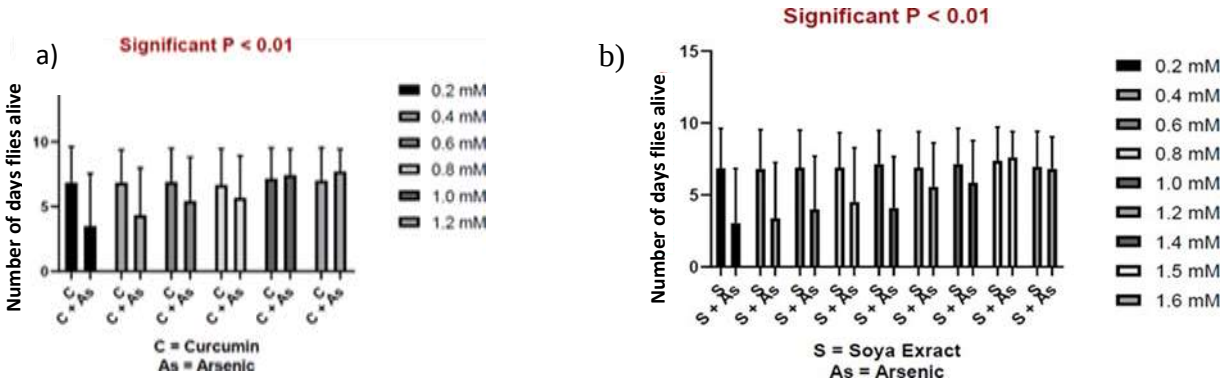

Fig 4. Number of days flies survived in (a) arsenic medium containing curcumin and (b) soya extract respectively

Table 3. Comparison of lifespan of *D. melanogaster* in different media

ARSENIC 0.5 milimoles					ARSENIC(0.55 mM) + CURCUMIN (1.0 mM)				ARSENIC (0.55 mM) + SOYA EXTRACT (1.5 mM)			
DATE	SET 'A'	SET 'B'	SET 'C'	MEAN ± SE	SET 'A'	SET 'B'	SET 'C'	MEAN ± SE	SET 'A'	SET 'B'	SET 'C'	MEAN ± SE
18.10.2021	10	10	10	10±0	10	10	10	10±0	10	10	10	10±0
19.10.2021	10	10	9	9.67±0.27	10	10	9	9.67±0.27	10	9	9	9.33 ± 0.27
20.10.2021	8	9	8	8.33±0.27	8	9	8	8.33±0.27	8	9	8	8.33 ± 0.27
21.10.2021	**8**	**7**	**7**	**7.33 ± 0.27**	**8**	**9**	**8**	**8.33±0.27**	**8**	**8**	**7**	**7.67 ± 0.27**
22.10.2021	6	5	6	5.67±0.27	8	7	7	7.33±0.27	7	7	7	7.00 ± 0
23.10.2021	6	5	6	5.67±0.27	7	7	5	6.33±0.54	6	6	6	6.00 ± 0
25.10.2021	**6**	**5**	**4**	**5±0.47**	**6**	**6**	**5**	**5.67±0.27**	**5**	**6**	**4**	**5.00 ± 0.47**
26.10.2021	5	4	4	4.33 ± 0.27	5	6	3	4.67±0.71	3	5	2	3.33 ± 0.71
27.10.2021	3	3	3	3 ± 0	3	4	2	3±0.46	1	3	1	1.67 ± 0.54
28.10.2021	1	2	1	1.33 ± 0.27	2	2	2	2±0	ALL DEAD	2	ALL DEAD	0.67 ± 0.54
29.10.2021	ALL DEAD	1	ALL DEAD	0.33 ± 0.27	ALL DEAD	1	1	0.67±0.11	ALL DEAD	ALL DEAD	ALL DEAD	0.33 ± 0.27
30.10.2021	ALL DEAD	ALL DEAD	ALL DEAD	~	ALL DEAD	ALL DEAD	ALL DEAD	~	ALL DEAD	ALL DEAD	ALL DEAD	~

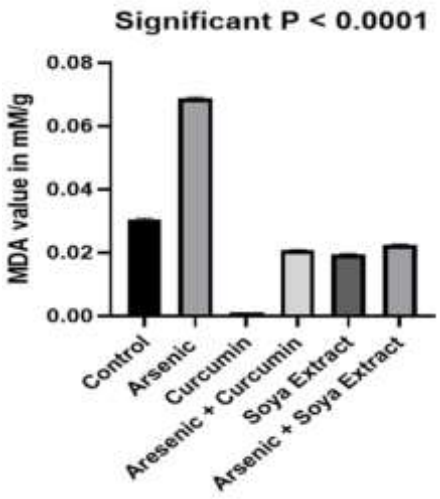

Fig 5: MDA values of flies cultured in different medium