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Effect of alloxan on the locomotor ability and developmental stages of *Drosophila melanogaster* (Oregon-R)

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Abstract

Background: Various bleaching agents are used in food industries among which some reacts to form alloxan. Therefore, the alloxan can indirectly enter a human body and thus form an important aspects for studying its effect on the development. In the present study, the effect of alloxan was studied on the climbing ability, pupation and emergence of flies. Fifty first instar larvae were introduced separately in the vials containing 0.001, 0.002, 0.003 and 0.004 M of alloxan. Then, the duration of pupation as well as the emergence of flies was noted each day till 20 days. The climbing assay was performed on the emerged flies.

Results: The results suggest that alloxan at 0.002, 0.003 and 0.004 M is potent in inducing the delay in pupation, emergence (of adult flies) and decreased locomotor activity of *Drosophila melanogaster*.

Conclusions: Alloxan exhibits toxic effects at 0.002, 0.003 and 0.004 M in *Drosophila*.

Keywords: Alloxan, *Drosophila*, Pupation, Emergence, Development

Background

Alloxan has structural similarity with glucose and selectively destroys the β-cells of pancreas (Bilic, 1975; Yasar et al., 2007). Due to this property it is widely used to induce diabetes (Type 1) in various experimental animals (Dhanesha et al., 2012; Skudelski, 2001). Alloxan has been reported to alter biochemical parameters of the liver, kidney functions, and histoarchitectural changes in various experimental animals (Yakuba et al., 2015). The low cost, rapid generation time and the availability of excellent genetic tools have made the fly important for basic research (Scott & Buchon, 2019). *Drosophila* has functional homologs of nearly 75% of the human disease genes and, therefore, is a good model to understand multiple pathways involve in the developmental process or in the progression of various human diseases (Panchal & Tiwari, 2017; Naz & Siddique, 2021). *Drosophila* has been used for the toxicological evaluations of various chemical compounds using cognitive, oxidative stress and genotoxic parameters (Adebambo and et al., 2020; Liu et al., 2019; Idda et al., 2020; Zhou et al., 2017; Siddique et al., 2013; Siddique et al., 2014; Siddique et al., 2015; Siddique et al., 2016). It is easy to perform experiments on fly as it renders fly embryo accessible to small molecules, toxicants and drugs (Rand et al., 2010).

The toxicity of alloxan at higher doses in humans cannot be ignored. In our earlier study on the third instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ Bg^{9})* alloxan has shown the toxic effects (Siddique et al., 2020). In this strain *Drosophila* heat shock gene, hsp70; fused to the *E. coli* β-galactosidase gene has been introduced into the *Drosophila* germline by the P- element microinjection method (Lis et al., 1983). Alloxan

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exhibited cytotoxicity and genotoxicity at 0.002, 0.003 and 0.004 M by generating reactive oxygen species (Siddique et al., 2020). Hence, we decided to study its effect on climbing ability, pupation and emergence (adult flies) of *Drosophila melanogaster*. Various bleaching agents (having oxide of nitrogen, chlorine, nitrosyl and benzoyl peroxide) in food industries react (chloride oxide with protein) to form alloxan (Shakila & Sasikala, 2012). *Drosophila* is a holometabolous insect and completes its entire life cycle within 2 weeks at 25°C (Ashburner & Thomson, 1978). Various external factors (temperature, humidity, exposure to various environmental agents) are known to influence the life cycle to different degrees (Podder & Roy, 2015). In the present study, we studied the effects of alloxan on the pupation, emergence (adult flies), and climbing ability of the emerged flies.

Methods

Fly strain

Drosophila melanogaster strain (Oregon-R) culture was maintained on the diet containing agar, corn meal, sugar and yeast at 24 ± 1°C (Nazir et al., 2003a, b). Alloxan (SRL, India) was dissolved in diet and the final concentrations of 0.001, 0.002, 0.003 and 0.004 M were established. The alloxan was mixed in diet during preparation to get the desired concentrations.

Effect on pupation and emergence of flies

Fifty first instar larvae were introduced in the vials containing the desired concentration of alloxan. The number of pupae followed by the number emerged flies were recorded in control as well as the treated groups till 20 days. Three sets of each treatment groups [including the untreated and positive control (MMS)] were employed in the study. From the 4th day, the numbers of larvae pupate followed by number of flies emergence were recorded separately and the data was expressed as the mean of three replicates (50 larvae/replicate) (Podder & Roy, 2015).

Drosophila climbing assay

The climbing ability or negative geotaxis measures the ability of the fly to climb up the walls of the vials (Pendleton et al., 2002). The climbing assay was performed according to the procedure described by Pendleton et al (2002). Ten flies (1–3 day old) were placed in an empty glass vial (10.5 cm × 2.5 cm). A horizontal line was drawn 8 cm above the bottom of the vial. After the flies had acclimated for 10 min at room temperature, both control and treated groups were assayed at random to a total of 10 trials for each. The mean values and standard error were calculated.

Statistical analysis

One way ANOVA post hoc Tukey was performed for statistical analysis using Statistica software (Statistica Soft Inc., USA).

Results

A significant day-wise difference in the rate of pupation (Fig. 1a) and emergence of flies (Fig. 1b) was observed at the exposure of 0.002, 0.003 and 0.004 M of alloxan compared to control (Fig. 1a and b; $p < 0.05$). The results obtained for the total number of larvae pupated showed a significant difference between control and the larvae exposed to 0.001, 0.002, 0.003 and 0.004 M of alloxan compared to control (Fig. 2a; $p < 0.05$). The results obtained for the total number of flies emerged showed a significant difference between control and the larvae exposed to 0.001, 0.002, 0.003 and 0.004 M of alloxan (Fig. 2b; $p < 0.05$). The flies emerged from the larvae exposed to 0.002, 0.003 and 0.004 showed a significant decrease of 1.17, 1.26 and 1.35 folds, respectively, in the climbing ability compared to control (Fig. 3; $p < 0.05$).

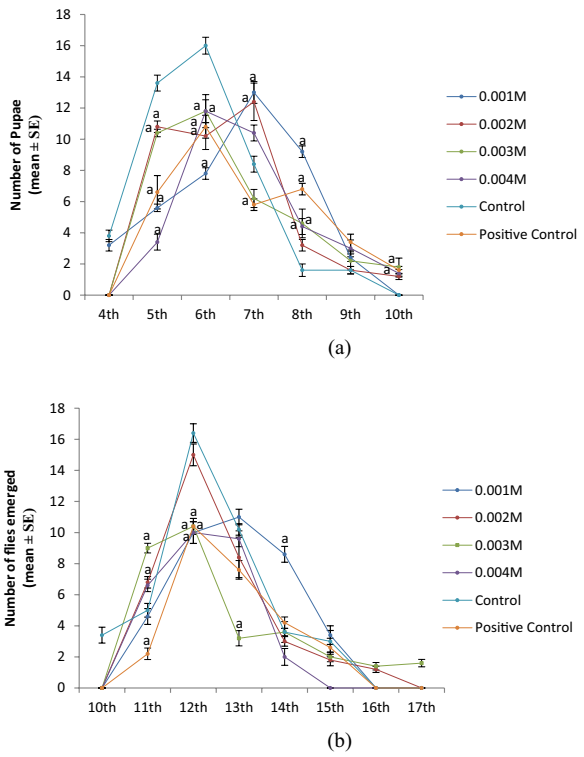

Fig. 1 Daywise distribution of the number of pupae observed **a** and the number of flies emerged **b** in the different treated and controls groups. ($n = 3$, 50 larvae/replicate; [a]significant at $p < 0.05$, compared to control). [A-Alloxan; A1-0.001 M; A2-0.002 M; A3-0.003 M; A4-0.004 M; PC-Positive Control, 0.1 µl/ml methylmethanesulphonate]

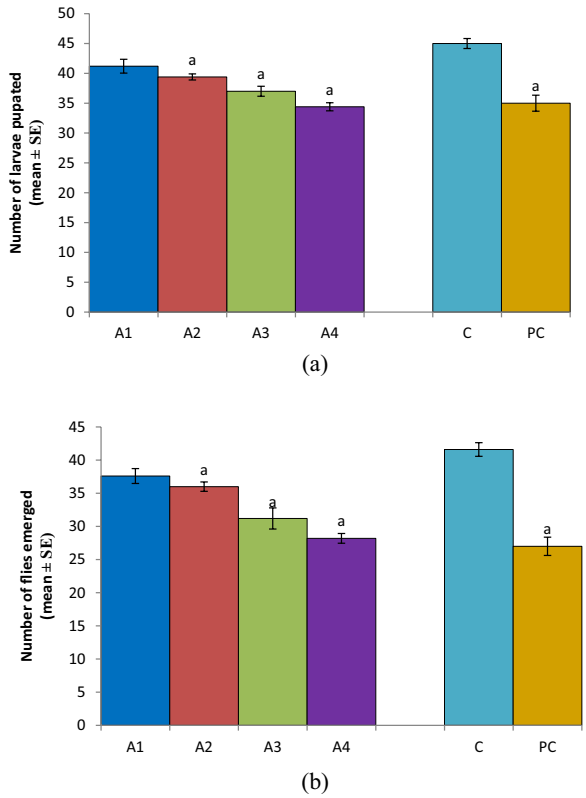

Fig. 2 Effect of alloxan on the number of larvae pupated **a** and flies emerged **b** ([a]significant at $p < 0.05$, compared to control). [$n = 3$, 50 larvae/replicate; A-Alloxan; A1-0.001 M; A2-0.002 M; A3-0.003 M; A4-0.004 M; PC-Positive Control, 0.1 µl/ml methylmethanesulphonate]

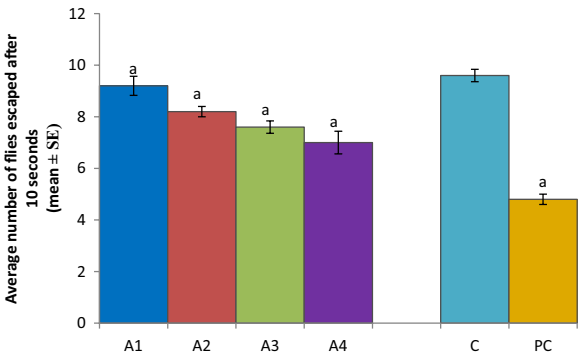

Fig. 3 Effect of alloxan on the climbing ability of flies in different treated and controls groups. ([a]significant at $p < 0.05$, compared to control). [$n = 3$, 10 flies/replicate; A1 = 0.001 M; A2 = 0.002 M; A3 = 0.003 M; A4 = 0.004 M; PC-Positive Control, 0.1 µl/ml methyl methanesulphonate

Discussion

The results of the present study showed that the exposure of alloxan not only affects the climbing activity of adult flies but also delayed the pupation and emergence of the flies. The studies with different insecticides and pesticides have shown well defined effects on the life cycle, hatch ability, and the emergence of *D. melanogaster* (Nazir et al., 2003a, b; Podder & Roy, 2015). The cytotoxicity by the alloxan involves the generation of reactive oxygen species (ROS) in the presence of intracellular thiols in a cyclic reaction with its reduction product as dialuric acid (Jorns et al., 1997). The rate of larvae transforming into pupae and pupa to adult reduced in a dose dependent manner. Every organism possesses a system for detoxification involving Phase I and Phase II enzyme changing the compounds in the form to be excreted by kidney (Benson & DiGiulio, 1992). The midgut of the insects is considered to be the most active region in the metabolism. The metabolism of imidacloprid occurs in the midgut of *D. melanogaster* larvae and the metabolites are rapidly excreted (Hoi et al., 2014). The metabolism of xenobiotics in vivo in insects is likely to be far more complex. As for various compounds especially, insecticides the metabolites have been reported to be more toxic than the parent compounds (Dunkov et al., 1997; Joussen et al., 2008). At higher dose the larvae failed to pupate and the mean number of pupae formed was significantly reduced. Similarly, the reduced rate of pupae becoming adults can be attributed to the interference in the function of the essential enzymes needed for the production of hormones involved in metamorphosis by alloxan at higher concentration (AL-Momani and Massadeh, 2005). The study conducted by Podder and Roy (2015) showed that the exposure of cryolite above a threshold value successfully deactivates the detoxifying enzymes, thereby allowing the toxicant to exert its effect on lengthening the pupal duration as well as the emergence of flies. The study of various compounds on *Drosophila* showed that either they lead to the massive production of ROS or disturb redox homeostasis leading to disturbed signalling pathway (Wu et al., 2021; Nazir et al., 2003a, 2003b; Beamish et al., 2021; Singh et al., 2020; Zou et al., 2016; Riddiford & Ashburner, 1991; Gao et al., 2020). Our earlier study on alloxan has shown the cytotoxic and DNA damage potential of the alloxan on third instar larvae of transgenic *Drosophila melanogaster (hsp70 lacZBg^{9})* (Siddique et al., 2020). The toxic effects were due to the increased oxidative stress and the generation of ROS (Siddique et al., 2020).

Conclusions

In our present study the reduction in the climbing ability of the emerged flies and the delay in the pupation as well as emergence of adult flies clearly demonstrates that alloxan is potent in disturbing the locomotory and developmental patterns in flies.

Abbreviations
MMS: Methyl methanesulphonate; ROS: Reactive oxygen species.

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Significance statement
Our earlier study has shown the toxic effects of alloxan in the third instar larvae of transgenic Drosophila. Various bleaching agents are used to make flour clean among which some reacts to form alloxan. In this context the present study demonstrates that the alloxan is also potent in inducing the developmental defects in Drosophila.

Author contributions
YHS design and performed experiments; MSA, FN and R performed experiments; YHS, MF and SP wrote manuscript; MSA, FN, R and SJ performed statistical analysis. All authors read and approved the final manuscript.

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Availability of data and materials
The data and the materials will be made available on request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declared no potential competing interest with respect to the research, authorship, and/or publication of this article.

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