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Effect of Sodium azide on larval tissues of a dipteran fly, *Sarcophaga ruficornis* (Sarcophagidae)

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ABSTRACT
The study highlights the adverse effects of Sodium azide on tissues in larvae of *Sarcophaga ruficornis*. In the form of the internal tissue damage Trypan blue dye exclusion assay is most commonly used to identify the tissue damage due to various chemical stresses. The intensity of tissue damage increases with an increase in the concentration of the test chemical.

Keywords: Chemical stress, Sodium azide, Tissue damage, Trypan blue, *Sarcophaga ruficornis.*

1. Introduction
The effect of various chemical stress on tissues has been studied with the help of trypan blue dye only in a few dipterans (Table -1).

Table 1: Tissue damage by miscellaneous chemicals in Diptera.

Name of Dipterans	Chemicals	References
Drosophila melanogaster	Methyl methanesulfonate	[1]
Drosophila melanogaster	Cypermethrin	[2]
Transgenic *Drosophila melanogaster*	L-Ascorbic acid	[3]
Transgenic *Drosophila melanogaster*	Nuvan and Dimecron	[4]
Transgenic *Drosophila melanogaster*	Dichlorvos and Chlorpyrifos	[5]
Transgenic *Drosophila melanogaster*	Argemone oil	[6] ,[7]
Transgenic *Drosophila melanogaster*	Effluents of Chrome plating industry	[8]
Transgenic *Drosophila melanogaster*	Captan	[9]
Transgenic *Drosophila melanogaster*	Hexachlorocyclohexane, Pentachlorophenol, Endosulphan	[10]
Lucilia cuprina	Endosulphan and monocrotophos	[11]
Musca domestica	Endosulphan and Monocrotophos	[12]
Musca domestica	Mercury	[13]

Trypan blue dye exclusion assay is one of the most commonly used method for measurement of cell viability in various medical and other fields. At physiological pH, this macromolecular stain is actively excluded from viable cells, but it readily enters cells in which membrane integrity has been compromised, and thus stains only dead or moribund cells [14]. Staining facilitates the visualization of cell morphology. In the present study the effect of sodium azide, a toxic compound, on the larval tissues of *S. ruficornis*, has been analysed by trypan blue dye, to assess tissue damage.

2. Material and Methods
Sarcophaga ruficornis Fab. (Sarcophagidae: Diptera) was reared in the laboratory at room temperature (26±2 °C) [15].The response to chemical stress on larval tissues was observed by performing *in vivo* chemical treatment and examined by staining with trypan blue.

2.1. Chemical Treatment

Five sets of 10 late third instar larvae were incubated in insect saline containing sodium azide in a test tube covered with muslin cloth and kept at room temperature (26±2 ºC). For control 10 late third instar larvae were incubated in insect saline in a test tube covered with muslin cloth and kept at room temperature (26±2 ºC). The concentration and time of exposure for chemical stress are presented in Table 2.

Table 2: Concentration and time of exposure for Sodium azide.

S.No.	Name of Chemicals	Concentration	Time (Minutes)	
1.	Sodium azide	20 mM	30	60
		30 mM	30	60
		40 mM	30	60

2.2 Trypan blue staining

After chemical treatment, control and treated larvae were dissected and immersed in 0.2 mg/ml Trypan Blue in Phosphate Buffer Saline (PBS) (pH 7.4) and rotated for 30 min. at room temperature. Tissues were rinsed three times in PBS, washed for 30 min. in PBS and immediately observed under dissecting microscope for Trypan Blue staining of tissues. Scoring of stained damaged tissues is according to the method of Krebs and Feder [16].

2.3. Mortality of larvae after chemical treatment

In the present study we also assessed the capacity of *Sarcophaga* to survive after chemical treatment. After each treatment, larvae were thawed at room temperature and the mortality rates were assessed 1 h after thawing. Moving larvae were designated as survivors and rest were designated as dead. Mortality rate was observed after chemical treatment for different time intervals i.e. 15, 30, 45 and 60 min. The rate of mortality increased with treatment time.

3. Observation: The staining patterns of different larval tissues after chemical treatment with Sodium azide are summarized in Table 3.

Table 3 -Staining pattern of control and stressed larval tissues of *S. ruficornis.*

	Bg	Sg	Gc	Mg	Hg	Mt
Control	+	-	+	-	+	+
(20mM/30 min.)	++	++	++	++	++	++++
(20mM/60 min.)	++	++	++	+++	+++	++++
(30mM/30 min.)	++	++	+++	+++	+++	++++
(30mM/60 min.)	++	++	+++	+++	+++	++++
(40mM/30 min.)	++	++	+++	++++	+++	++++
(40mM/60 min.)	++	++	+++	++++	+++	++++

Bg-Brain ganglia, Sg - Salivary gland, Gc - Gastric caeca, Mg - Mid gut, Hg –Hind gut , Mt - Malpighian tubule. (-)= No staining, (+) = Pale blue staining, (++) = Moderate staining, (+++) =Darker staining, (++++) = Darkest staining.

3.1. Tissue damaged in larvae after chemical stress

Sodium azide 20 mM treatment, for 30 min. and 60 min. to the larvae shows a characteristic staining pattern in brain ganglia, salivary gland, gastric caeca, mid gut, hind gut and malphighian tubule. All the tissues show darker staining as compared to control. The staining pattern with the treatment of 20 mM Sodium azide, in brain ganglia, salivary gland and gastric caeca show moderate staining, while mid gut and hind gut reveal darker staining. Malphighian tubule shows the darkest staining.

Chemical treatment at 30 mM concentration for 30 min. and 60 min., elicits a response which is similar to 20 mM Sodium azide treatment, in brain ganglia, salivary gland and malpighian tubule, while the gastric caeca, mid gut and hind gut show darker staining, as compared to 20mM sodium azide treatment. As the time and concentration of sodium azide increased i.e.40mM for 30 min.and 60 min. similar finding were observed. The intensity of staining was strongest in midgut and malpighian tubules it means these tissues show lowest tolerance to chemical stress.

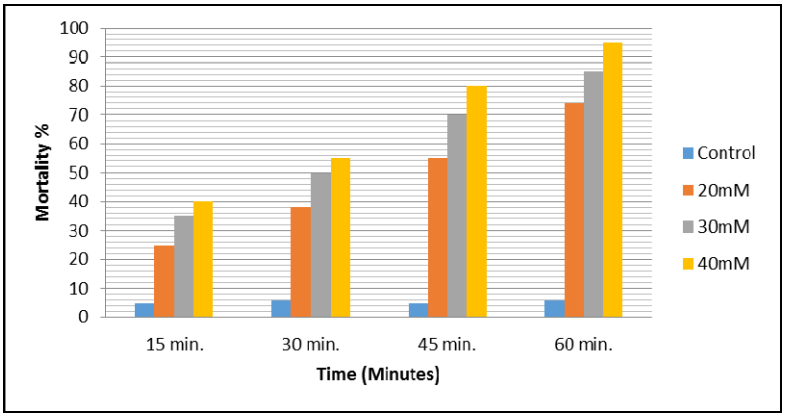

Fig 1: Effect of Sodium azide on mortality of *S. ruficornis* larvae.

3.2. Mortality rate after Sodium azide treatment

The rate of mortality increased with treatment time, though it was low at 20 mM (15 min.). After 60 min. exposure to chemical treatment the mortality is approximately 100%. The mortality rate of *S. ruficornis* larvae after chemical stress is represented in Fig.1.

4. Discussion

The general stress response involves the expression of stress protein i.e. Heat Shock Protein (Hsp). Their induction is often accompanied by tolerance to these stresses [17-22]. There is a close link between stress protein expression and tissue damage as revealed by trypan blue staining [23].

Krebs and Feder [16] opined that gut tissues show extensive necrosis after heat stress as revealed by trypan blue staining which increased in all gut tissues *viz*.,salivary gland,gastric caeca, mid gut, hind gut as compared to nongut tissues. In *Sarcophaga ruficornis* larvae the gut tissues *viz*. gastric caeca, midgut, hindgut and malpighian tubules are more susceptible to chemical stress as compared to non gut tissues i.e. brain ganglia and salivary gland. As these tissues darkly stained with Trypan blue dye after Sodium azide treatment as compare to control. Moreover as the concentration of chemical increases the tissue damage is also increases.

In our opinion that in *S. ruficornis* there is a tissue specific expression of heat shock protein because in earlier studies also it was found that brain ganglia and salivary gland have high tolerance and gut tissues have low tolerance to heat and cold stress[24]. It might be concluded that in *S. ruficornis* larvae the heat shock proteins are early expressed in non gut tissues than the gut tissues.

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