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A study of anti-stress property of *Convolvulus pluricaulis* (Shankhapushpi) on stress induced *Drosophila melanogaster.*

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Abstract: Anti-stress property of *Convolvulus pluricaulis* has been evaluated using *D. melanogaster*. Four groups of flies were reared simultaneously under similar conditions. The first

group of flies (Control) were reared on normal media, the second on the media containing methotrexate, the third group on the media containing methotrexate and 0.5 gm of plant sample (commercially available). The last group of flies was reared on the media containing only 0.5 gm of plant sample. Then the flies were subjected to the assay of stress related marker enzymes like superoxide dismutase (SOD) and catalase. Then the activities of enzymes were compared with each group of flies. Taken together, our data provide scientific support for the anti-stress property of *C. pluricaulis.*

Introduction

Living in an oxygenated environment has required the evolution of effective cellular strategies to detect and detoxify metabolites of molecular oxygen known as reactive oxygen species (Finkel and Niki, 2000). Oxidative stress occurs, however, if noxious oxygen derivatives are not controlled by antioxidant defense systems (Sies, 1985). One physiological response to stress is the increased activity of certain enzymes (Sorensen *et al.,* 2003).

The burden of ROS production is largely counteracted by an intricate antioxidant defense system that includes the enzymatic scavenges like SOD, catalase, and glutathione peroxides. SOD speeds the conversion of superoxide to hydrogen peroxide, whereas catalase and gluthathione peroxidase convert hydrogen peroxide to water. In addition to these well characterized antioxidant enzymes, at least five members of a new family of peroxidase scavengers, termed peroxiredoxins, has recently been isolated (Chae *et al.,* 1999). Both SOD and catalase are the indispensable enzymes for aerobic creatures, because of their roles in scavenging the reactive oxygen intermediates. Environmental factors, particularly oxidative stress, influences the level of these enzymes in biospecies along with the innate nature of cells. A majority of the diseases or disorders are linked to oxidative stress due to the free radicals (Tiwari, 2001). An answer to this perplexing problem of countering stress induced perturbations of physiological homeostasis came from the plant kingdom (Carlini, 2003).

Methotrexate decreases titers of reduced folates, interferes with DNA synthesis, and results in the arrest of rapidly proliferating cells; it results in stress in *Drosophila* flies (Barclay *et al*., 1982; Joslynn *et al.,* 2005). *D. melanogaster* was reared on the media containing different concentration of methotrexate to induce stress. There was an increase in the activity of SOD and catalase in flies.

To obtain an insight into the anti-stress property of *Convolvulus pluricaulis*, the flies were reared on the media containing different concentrations of methotrexate along with 0.5 gm of the plant sample. The decreased activity of SOD and catalase was observed. Another group of flies was reared on the media containing only 0.5 gm of the plant sample in which the activity of SOD was decreased and the activity of catalase was slightly increased. These findings set the stage to conclude that the plant sample may have antitheses property in it.

Materials and Methods

Fly rearing

Flies were reared on "Cream of Rava-Agar" media at $26^{o}C \pm 1^{o}C$. The "DROSOPHILA STOCK CENTER" Dept of Zoology, University of Mysore provided the stocks of wild type of *D. melanogter*. The flies reared on this medium served as control flies.

Exposure to Methotrexate

To induce stress in *D. melanogaster*, methotrexate was added into the normal medium in different concentrations (5, 10, 15, 20, 25 ppm) in different rearing bottles. Then 5 male and 5 female normal flies were transferred to these media and allowed to multiply. The marker enzyme activities were assayed to confirm the induction of stress.

Treatment with the plant sample

To test the anti-stress property of the plant sample (*Convolvulus pluricaulis*), along with the different concentrations of methotrexate, 0.5 gm of plant sample was added to the normal media. Then 5 male and 5 female normal flies were transferred to this medium and allowed to multiply. Catalase and SOD activities were assayed.

Five males and 5 females were transferred to the medium containing only 0.5 gm of plant sample and the enzyme assay was carried out.

SOD activity assay

SOD (EC 1.15.1.1) was assayed (Beauchamp and Fridovich, 1971) in each group of flies at a time. Five flies from each group were homogenized in 200 microliter of 250 mM Phosphate buffer (pH 7.8), centrifuged at 12000 rpm for 10 min at 4^{o}C. Supernatant (enzyme extract -0.1 ml) was mixed with cocktail of solutions containing 250 mM phosphate buffer (0.8 ml), 100 mM methionine (1 ml), 100 mM riboflavin (0.5 ml), 5 mM EDTA (0.1 ml),750 mM NBT (0.05 ml). The volume was made up to 3 ml with distilled water. The cocktail solution without the supernatant (enzyme) and NBT was prepared to serve as a blank. Control solution was prepared with the presence of NBT, but no enzyme. Reduced NBT to formazone was read at 560 nm. The protein content of supernatant was estimated by Lowry's method. The activity was expressed in units /mg of protein.

Catalase activity assay

Catalase (EC 1.11.1.6) was assayed (Beers and Sizer, 1952) in different group of flies at a time. As in the SOD assay, here also five flies from each group were homogenized in 200 microliter of 50 mM phosphate buffer (pH 7.0) and centrifuged at 10000 rpm for 10 min at 4^{o}C. Supernatant (enzyme extract) was mixed with 2.9 ml of 30% hydrogen peroxide (freshly prepared using 50 mM phosphate buffer). Decrease in the absorbance due to decomposition of hydrogen peroxide was monitored at 240 nm in a spectrophotometer. Protein content of supernatant was estimated by Lowry's method. The activity was expressed in units /mg of protein.

Results

Increased SOD and Catalase activity in flies exposed to methotrexate

Rearing of flies on media containing methotrexate resulted in the increased activity of SOD and Catalase. These enzymes are the marker enzymes for the oxidative stress. The activity of SOD and Catalase increases, with respect to the increased concentration of methotrexate in the media (Figures 1 and 2), when compared to control flies on normal media (methotrexate absent, *i.e*., 0 ppm).

Enzyme activity in the stress induced flies treated with plant sample

We found the decreased activity of enzymes in flies reared on the media containing different concentrations of methotrexate in the presence of plant sample. The elevated level of enzyme due to methotrexate was decreased in the presence of plant sample (Figures 3 and 4).

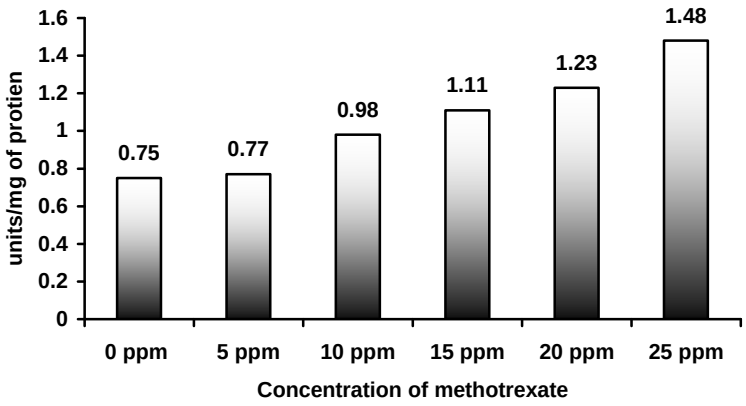

Figure 1. Activity of SOD increases gradually in flies reared on media containing different concentration of methotrexate when compared with control flies.

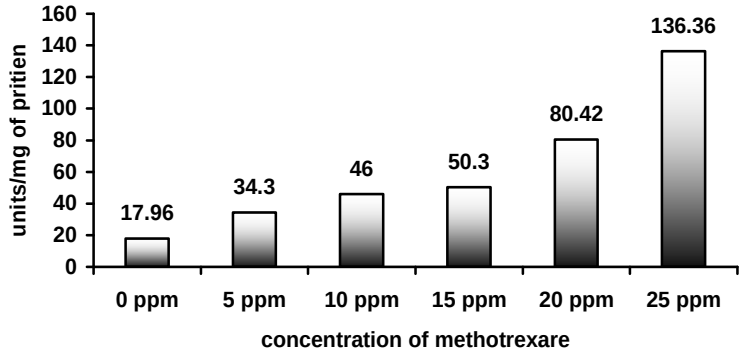

Figure 2. Activity of catalase increases gradually in flies reared on media containing different concentration of methotrexate when compared with control flies.

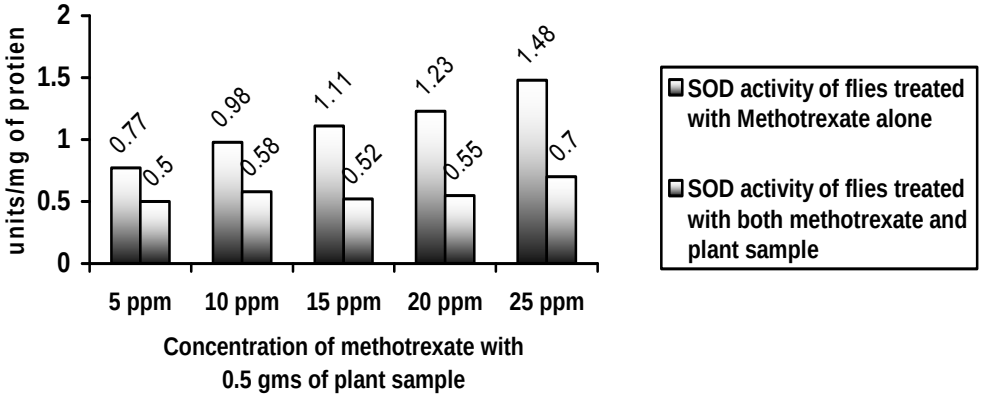

Figure 3. The increased activity in stressed induced flies is reduced when treated with plant sample at a constant range.

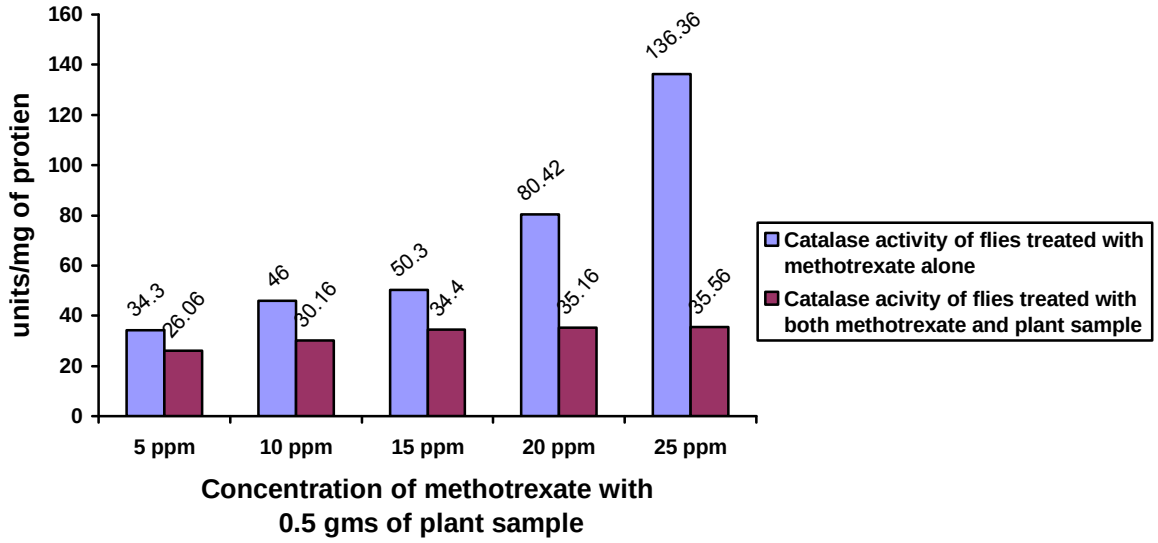

Figure 4. Similarly the increased activity of Catalase in *D. melanogaster* is also decreased by the plant sample.

Variation of enzyme activity in normal flies treated with plant sample alone

Difference in the enzyme activity was observed when the flies were reared on the media containing only 0.5 gm of plant sample. There was a decreased SOD activity (Figure 5) and the catalase activity was slightly increased (Figure 6) when compared with the control flies.

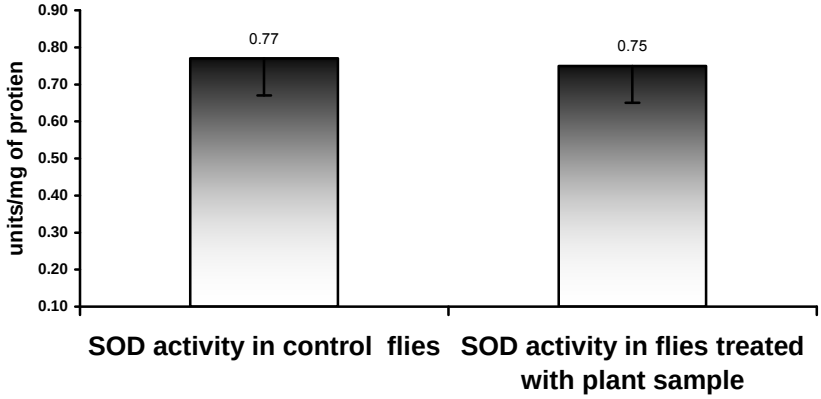

Figure 5. Compared with the activity of the control flies, the activity in flies treated with plant sample alone was decreased.

Discussion

Long-term exposure to multiple stressors can cause depression in humans. Induction of depression using CMS is considered as the most congruent animal model of depressive conditions observed in humans after long term exposure to multiple stressors (Willner, 1986). The results of the current study demonstrated decreased activity of the stress related marker enzymes in stress induced *Drosophila melanogaster* flies.

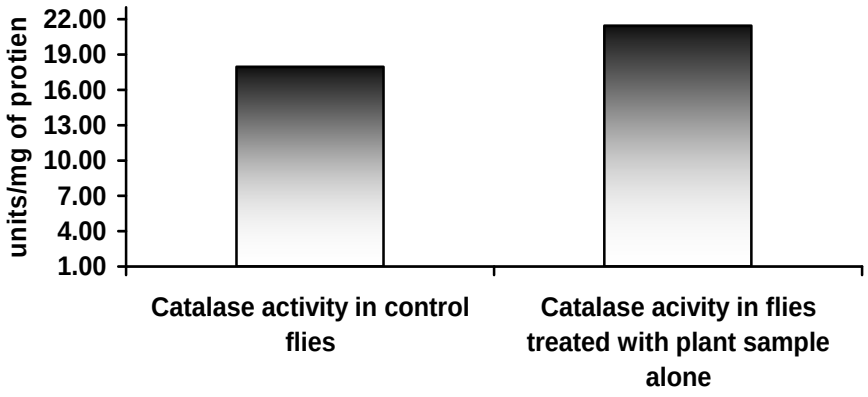

Figure 6. Compared with the activity of the control flies, the activity in flies treated with plant sample alone was slightly increased.

SOD dismutases the highly reactive superoxide anion to the less reactive species H_2O_2 (Teixeira *et al*., 1998). Catalase, a haeme-containing enzyme, scavenges hydrogen peroxide to water and molecular oxygen (Mates and Sanchez-Jimenez, 1999), and non-enzymic ascorbic acid, which is a water-soluble antioxidant forage free radical protect the biological system from oxidative stress (Beyer, 1994).

When flies were treated with plant sample alone the activity of SOD was decreased and the activity of catalase was increased. The increase in the catalase activity may be because of the additional components present in the plant sample, since the plant sample used was commercially available crude sample of *C. pluricaulis*, and hence further study has to be achieved to isolate active constituents from the plant that can be used for applied research. Taken together our data suggest that the plant sample we used may have anti-stress property in it.

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