Anti-stress property of *Rauwolfia serpentine* (Sarpagandha) on stress induced *Drosophila melanogaster.*

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Abstract

Stress is a condition or circumstance (not always adverse), which can disturb the normal physiological and psychological functioning of an individual. In medical parlance `stress` is defined as a perturbation of the body`s homeostasis. This demand on mind-body occurs when it tries to cope with incessant changes in life. A `stress` condition seems `relative` in nature. Anti-stress property of *Rauwolfia serpentina* (Sarpagandha) was evaluated using the *Drosophila melanogaster* (fruit fly) as a model organism. In the first group, control flies were taken as normal flies and were considered as control to compare with that of second group of stress induced flies by different concentrations of MTX, and a third group of flies were reared on media containing plant sample of 0.1g along with MTX, and the last group of flies were reared on the media containing only 0.1g of plant sample. Then the flies were subjected for enzymatic assay using enzymes Catalase and SOD at a time. The result of the present study showed that the plant powder used may have the anti-stress property as it reduced the stress, which was demonstrated by the reduced activities of marker enzymes like SOD and Catalase in stress induced *Drosophila melanogaster.*

Introduction

Life in the 21st Century is infinitely far more complex than it has ever been. It had been never designed to live in this complex, modern world with its many demands. One physiological response to stress is the increased activity of certain enzymes (Sorensen *et al.,* 2003). Oxidative stress is a "privilege" of aerobic organisms. It can be induced by endogenous and exogenous factors (Dallman *et al.,* 2005).

Overproduction of the reactive oxygen species (ROS) superoxide (O_2-) and hydrogen peroxide (H_2O_2) are increasingly implicated in human disease and aging. ROS are also being explored as important modulating agents in a number of cell signaling pathways. Increasing attention has been devoted to developing catalase or peroxidase mimetic as a way to treat overt inflammation associated with the pathophysiology of many human disorders.

It focused on recent development of catalytic scavengers of peroxides and their potential use as therapeutic agents for pulmonary, cardiovascular, neurodegenerative, and inflammatory disorders. Antioxidants, the free radical scavengers, however, are shown to be anticarcinogens. They function as the inhibitors at both initiation and promotion/transformation stage of carcinogenesis and protect cells against oxidative damage (Sun *et al.,* 1993).

The novel antioxidant enzyme was shown to reduce hydro peroxides and, more recently, peroxynitrite with the use of electrons provided by a physiological thiol like thioredoxin. 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).

Their defense against these free radicals is achieved by natural antioxidant molecules but also by antioxidant enzymes. Three important anti-oxidant enzymes are Cu/Zn- superoxide dismutase (Cu/Zn-SOD), catalase, and selenium-glutathione peroxidase. They are all necessary for the survival of the cell even in normal conditions. In addition, these three enzymes act in a cooperative or synergistic way to ensure a global cell protection. However, optimal protection is achieved only when an appropriate balance between the activities of these enzymes is maintained (Michiels *et al.,* 2005).

Reactive oxygen species (ROS) are defined as oxygen-containing species that are more reactive than $O(_2)$ itself, which include hydrogen peroxide and super oxide. Although these are quite stable, they may be converted in the presence of transition metal ions, such as Fe (II), to the highly reactive oxygen species (hROS). hROS may exist as free hydroxyl radicals (HO), as bound ("crypto") radicals or as Fe(IV)-oxo (ferryl) species and the somewhat less reactive, non-radical species, singlet oxygen (Tiwari *et al.,* 2001).

Catalytic activity is present in nearly all animal cells and organs and in aerobic microorganisms. Catalase activity varies greatly between tissues with highest activities in the liver, kidney, and erythrocyte, and lowest activity present in connective tissues. In eukaryotic cells the enzyme is concentrated in sub-cellular peroxisome organelles.

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long (Boon *et al.,* 2007). The optimum temperature also varies by species.

Catalase exhibits an unusual kinetic behavior in that it is not possible to saturate the enzyme with H_2O_2 substrate up to 5M catalase concentration, but there is a rapid inactivation of the enzyme at substrate concentrations above 0.1M H_2O_2. Therefore, the activity assay is typically carried out with 10-50 mM H_2O_2. Since H_2O_2 substrate must be present at substantially less than saturated concentration, the enzyme activity is dependent on precise concentration of H_2O_2.

Stress was induced to the flies by adding Methotrexate to check the anti-stress property of the plant. Methotrexate is a structural analog of folic acid and acts by binding and inhibiting dihydrofolate reductase (DHFR), a key enzyme required for intracellular folate metabolism. It is an antimetabolite and antifolate drug, and works by inhibiting the metabolism of folic acid. Methotrexate acts specifically during DNA and RNA synthesis and is cytotoxic during the S-phase of the cell cycle. Methotrexate is commonly used in combination with misoprostol to terminate early pregnancies, *i.e*. pregnancy in the early stages. It may also be used in case of missed miscarriage, in which fetal demise has occurred, but the body has not expelled the fetus (Mol *et al.,* 2008).

Reduced folate is involved in normal synthesis and metabolism of neurotransmitters in central nervous system. Methotrexate may exert a beneficial effect in psoriasis by mechanism other than inhibition of dihydrofolate reductase. Methotrexate inhibits neutrophil chemotaxis (Johnston *et al.,* 2005).

Addition of folic acid to methotrexate therapy should allow dermatologists to use methotrexate in a much better way and enhance patient compliance (Mol *et al.,* 2008). The improved efficacy of high-dose methotrexate as compared to conventional dose methotrexate suggests that osteosarcoma may have intrinsic methotrexate resistance, which can be Sarcoma overcome by achieving a high extracellular drug concentration (Johnston *et al.,* 2005). Intracellular methotrexate undergoes polyglutamylation whereby the polyglutamylated methotrexate is preferentially retained in the cell and ultimately results in DHFR inhibition (Bertino, 1993). As a cytotoxic drug it may slow the rapid growth of cells in the synovial membrane that lines the joints (Sirotnak, 1985). Methotrexate is a chemotherapy drug used to treat leukemia, lymphomas, and osteosarcoma. It is also used in the treatment of AIDS and rheumatoid arthritis. Analogues of folic acid were in development, and by 1950, methotrexate (then known as ametopterin) was being proposed as a treatment for leukemia (Meyer *et al.,* 1950).

The rates of termination for ineffectiveness were lower and the adjusted drug retention rates were better for re-employed courses. With regard to new treatment strategies, including monitoring, co-medication and even the increasingly employed paradigm to change therapy if a state of low disease activity is not reached within few months.

Materials and Methods

The *DROSOPHILA* STOCK CENTRE, Department of Zoology, University of Mysore, provided the stocks of wild type of *D. melanogaster*. Further the stocks were cultured in our laboratory at 26°C. As the temperature decreases, the development time increases (Ashburner and Thompson, 1978; Ashburner *et al*., 2005). At higher temperature around 31 degrees, flies may become sterile and may result in death. They require a controlled temperature and humidity environment. Stocks kept at room temperature were transferred to fresh media every 20 days or the flies (5 male and 5 female flies) were transferred to fresh media when overcrowding occurs.

Culturing of stress induced flies

Methotrexate is an antimetabolite; it interferes with the way cells utilize essential nutrients, so this chemical agent was added to create stress. Into the bottle along with media, methotrexate was added in different concentrations in the range of 5ppm, 10ppm, 15ppm, 20ppm, and 25ppm. The catalase and SOD activity was increased in the flies cultured in the media containing MTX. This was confirmed by comparing the activity of the enzymes with the control flies cultured in normal media.

Enzyme collection

Different groups of flies were taken in different eppendroff tubes as methotrexate flies of different concentrations from 5ppm-25ppm and also theses stress induced flies along with plant sample. These were fully homogenized in a 200 microlitres of fresh phosphate buffer of 50 mM for catalase assay of pH 7.0 and for SOD assay 250mM phosphate buffer of pH 7.8. These were homogenized with the help of tissue homogenizer, which was kept in ice cold condition and centrifuged at 8000rpm for 20 min in a cooling microfuge. After centrifugation supernatant was transferred to fresh eppendroff tube, and 100 microlitres of this supernatant serves as enzyme source for both Catalase and SOD enzymatic assays.

Assay of catalase enzyme

Catalase enzyme (EC 1.11.1.6) activity method is essentially described by Beers and Sizer (1952). 2.9 ml of hydrogen peroxide was taken along with 0.1 ml of enzyme extract, then immediate mixing by inversion, and the absorbance was read at 240 nm in spectrophotometer, the absorbance decreases gradually. Then activity of Catalase was calculated. Then to know the specific activity, protein estimation was done which was expressed in units/mg of protein.

Assay of SOD

SOD enzyme (EC 1.15.1.1) was assayed using a slightly modified procedure originally described by Beauchamp and Fridovich (1971). Mix 3 ml of cocktail solution containing Phosphate buffer (0.8 ml), Methionine (1 ml), riboflavin (0.5 ml), EDTA (0.1 ml), NBT (0.5 ml), and the volume is made up to3 ml by adding distilled water. A blank was set without the enzyme and NBT to calibrate the spectrophotometer having buffer (1.0 ml), Methionine (1 ml), riboflavin (0.7 ml), and EDTA(0.3 ml). Another control was prepared having NBT but no enzyme and is taken as a reference

control, which contains buffer (0.9 ml), Methionine (1 ml), riboflavin (0.5 ml), EDTA (0.1 ml), and NBT (0.5 ml). These colored solutions absorbance was read at 560 nm immediately to know the activity, and later on to know the specific activity protein estimation was done by Lowry's method and units expressed in Units/mg of protein.

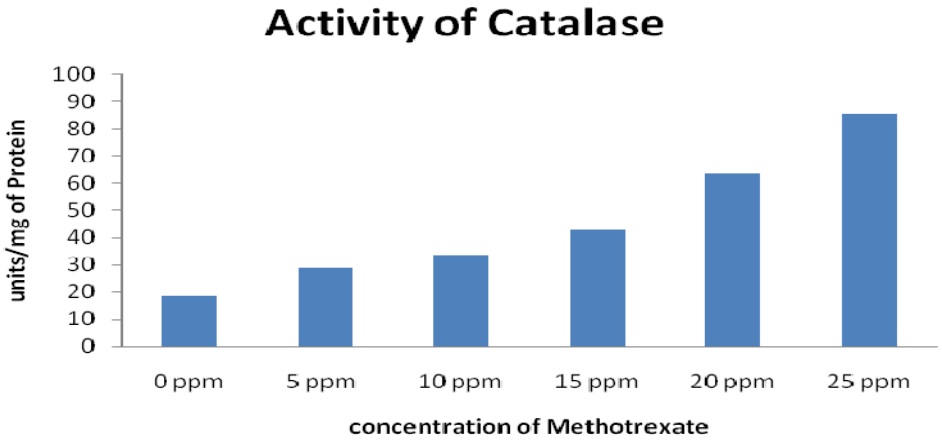

Figure 1. Activity of catalase in flies reared on media containing different concentrations of methotrexate when compared with control flies.

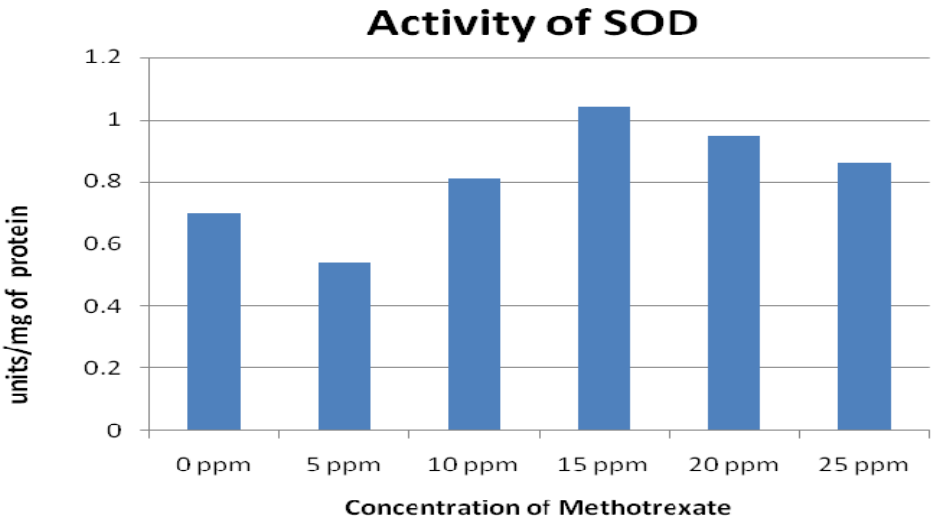

Figure 2. Activity of SOD in flies reared on media containing different concentrations of methotrexate when compared with control flies.

Table 1. Increased Catalase and SOD activity in flies exposed to different concentration of Methotrexate

Concentration of MTX	0 ppm (Control)	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
Catalase Activity in units/mg of protein	18.32	28.84	33.43	42.69	63.49	85.72
SODActivity in units/mg of protein	0.70	0.54	0.81	1.04	0.95	0.86

Results

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

(Figure 1 and 2, Table 1), when compared to control flies on normal media (no Methotrexate, *i.e.*, 0 ppm).

Enzyme activity in stress induced flies treated with plant sample

The activity of SOD is decreased in flies, reared on the media containing different concentrations of Methotrexate in presence of plant sample. The elevated level of enzyme due to Methotrexate was decreased in the presence of plant sample (Figure 3 and 4, Table 2).

The enzyme activity was different in the flies reared on the media containing only 0.5 gm of plant sample. There was increased Catalase activity compared with control flies (Figure 5), and the SOD activity was found to be decreased when compared to the control flies (Figure 6, Table 3).

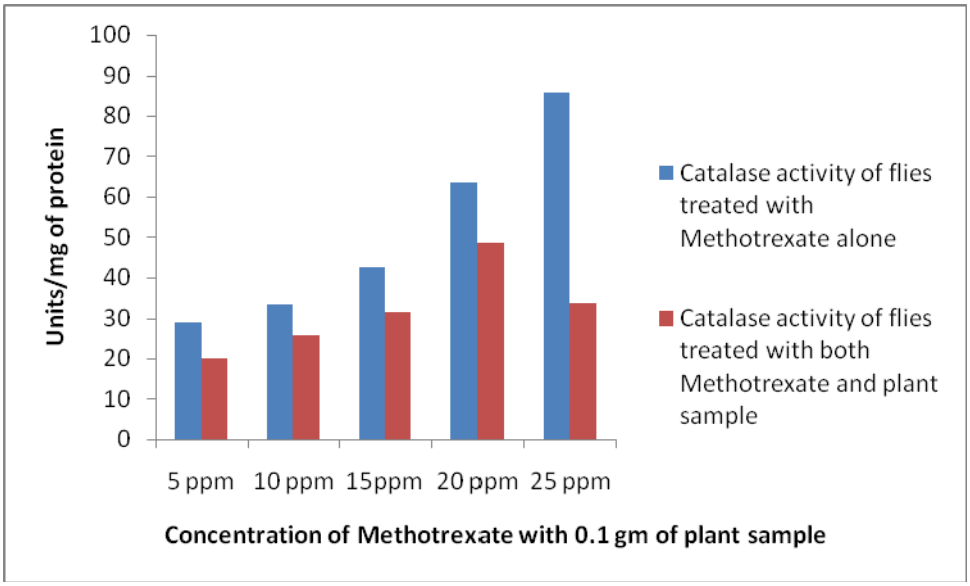

Figure 3. The increased activity of catalase in stressed induced flies is reduced when treated with plant sample.

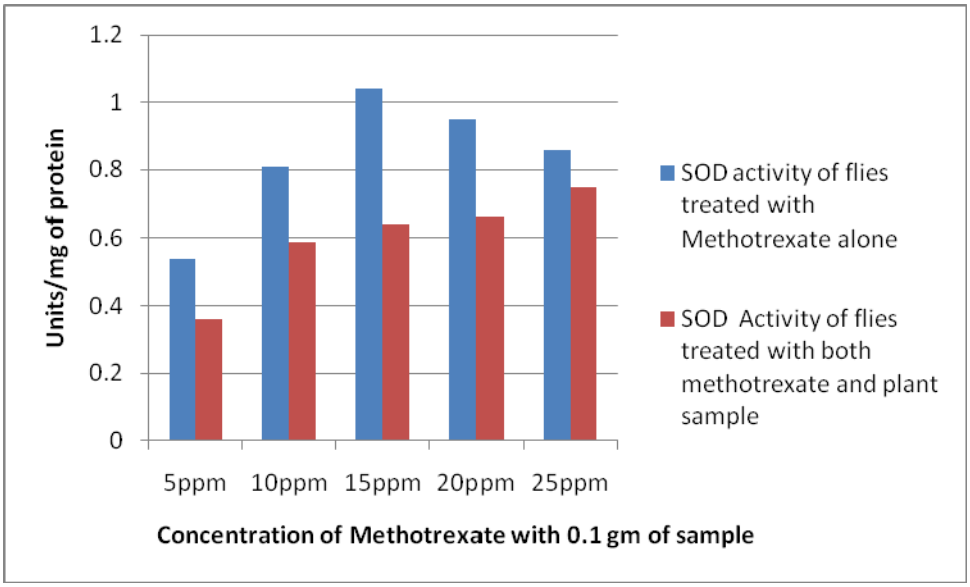

Figure 4. The increased activity of SOD in stressed induced flies is reduced when treated with plant sample.

Table 2. Catalase and SOD activity of alone stress induced flies and MTX + 0.1 gm of plant sample of different concentrations.

	Concentration of MTX	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
Catalase Activity in units/mg of protein	MTX alone	28.84	33.43	42.69	63.49	85.72
	MTX + 0.1gm plant sample	19.86	25.74	31.57	48.53	33.63
SOD Activity in units/mg of protein	MTX alone	0.54	0.81	1.04	0.95	0.86
	MTX + 0.1gm plant sample	0.36	0.59	0.64	0.66	0.75

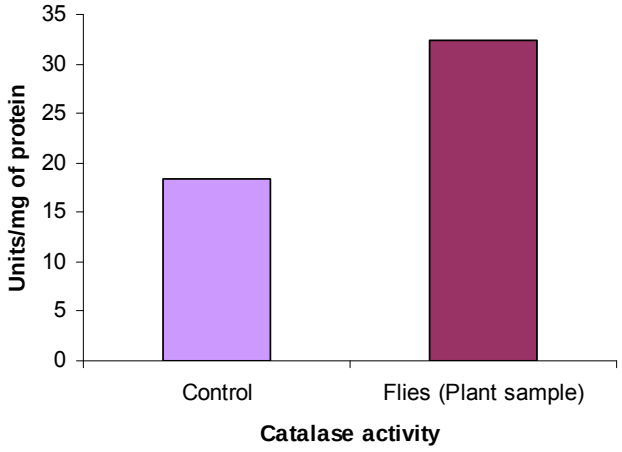

Figure 5. Catalase activity compared with the control flies, and the flies treated with plant sample.

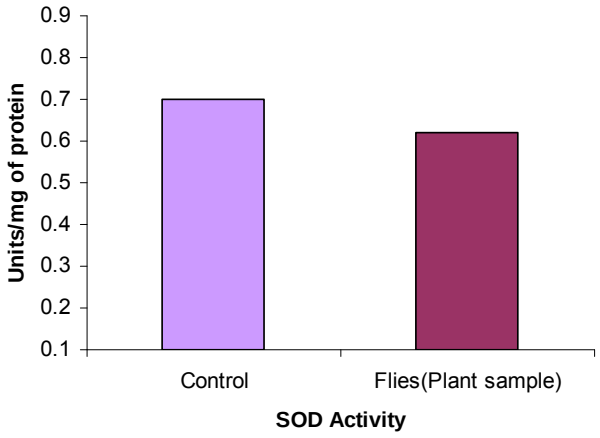

Figure 6. SOD activity compared with the control flies, and the flies treated with plant sample.

Table 3. Enzyme activity variation in normal *D. melanogaster* flies and flies treated with plant sample alone.

	Catalase Activity in units/mg of protein	SOD Activity in units/mg of protein
Control flies	18.32	0.70
Flies treated with plant sample alone	32.42	0.62

Discussion

Catalase and SOD activity was measured in stress induced flies along with the plant sample of 0.1 gm, but there was a decrease in the activity compared to the control flies. Oxidative stress has been implicated to play a role, at least in part, in pathogenesis of many disease conditions and toxicities in animals and overproduction of reactive oxygen species and free radicals due to use of toxic chemicals showed elevated increased catalase and SOD activity. So decrease in the catalase activity showing that plant sample is effective in decreasing catalase activity, but there was not much difference in the SOD activity compared to the control indicating that plant may not be found effective in decreasing SOD activity. 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 increased and the activity of catalase was decreased. The increase in the SOD activity may be because of the additional components present in the plant sample, since the plant sample used was commercially available crude sample of *R. serpentina*, 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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New records of Drosophilidae in southern Amazônia.

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Introduction

The records in the literature of Brazilian species of the family Drosophilidae show an evident concentration in the South, Southeast, and Center-West regions of the country (Val *et al*., 1981; Gottschalk *et al*., 2008). These records cover several environments and different resources for