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ORIGINAL ARTICLE

A study on anti-stress property of *Nardostachys jatamamsi* on stress induced *Drosophila melanogaster*

Shilpashree R. and Sathish Kumar B.Y.*

Postgraduate Department of Biotechnology, JSS College of Arts, Commerce and Science (Autonomous), Ooty Road, Mysore-25, Karnataka, India.

*E-Mail: **bysathish@yahoo.com**

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Stress is a feeling that's created when we react to particular events. It s the body's way of rising to a challenge and preparing to meet a tough situation with focus, strength, stamina, and heightened alertness. As a result of the stress immune system can be suppressed by chronic stress opening to increased infections and increasing the risk of autoimmune diseases. So one has to learn away to overcome stress. Here is an attempt made to overcome the stress induced in *Drosophila melanogaster* a model organism, in this study. Methotrexate is used to induce the stress at different concentration taking different group of flies and a *Nardostachys jatamamsi* plant extract having antistress property is used to relieve the stress induced. This stress relieve measured by the various stress related enzymes like catalase and Superoxide dismutase by this antistress property of the plant *Nardostachys jatamamsi* was shown.

Key words: Catalase / Drosophila / MTX (Methotrexate) / Stress / Superoxide dismutase

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Stress refers to the consequence of failure of organism, human or animal to respond appropriately to emotional or physical threats, whether actual or imagined Stress covers a wide range of phenomena from mild irritation to drastic dysfunction that may cause severe health breakdown (Selye, 1982). Stress can affect just about every body system. It affects digestive system resulting in stomach ache or diarrhoea. Cortisol increases appetite leading to weight gain. By products of cortisol acting as a sedatives, can lead to feeling of depression, anxiety, and helplessness.

In aerobic organisms the energy needed to fuel biological functions is produced in the mitochondria via the electron transport chain In addition to energy, reactive oxygen species (ROS) are produced which have the potential to cause cellular damage. ROS can damage DNA, RNA, and proteins which theoretically contributes to the physiology of ageing (Rada *et al.,* 2008).

According to the Free-radical theory, oxidative damage initiated by reactive oxygen species is a major contributor to the functional decline that is characteristic of ageing. While studies in invertebrate models indicate that animals genetically

engineered to lack specific antioxidant enzymes (such as SOD) generally show a shortened lifespan (as one would expect from the theory), the converse, increasing the levels of antioxidant enzymes, has yielded inconsistent effects on lifespan (though some well-performed studies in Drosophila do show that lifespan can be increased by the over expression of MnSOD or glutathione biosynthesizing enzymes). In mice, the story is somewhat similar. Deleting antioxidant enzymes generally yields shorter lifespan, though over expression studies have not (with some recent exceptions), consistently extended lifespan (Muller *et al.*, 2007)

Ageing is a natural process except under extreme conditions such as stress and grief. The constant stressors or stress conditions result in a loss in neural and hormonal balance this loss will cause increased oxidative damage accelerating ageing in our body, because chronic disturbances in body homeostasis ultimately affect our hormone secreting glands, cell repair and collagen in our skin and connective tissues. When our body is under stress reactive oxygen species are produced and these tend to damage the DNA, proteins, lipids etc leading to ageing and any diseases (Baker *et al.*, 1998) some of the defense enzymes such as catalase, superoxide dismutase (SOD), catalase the dismutation of superoxide into oxygen and hydrogen peroxide. Here in this study the increase in the stress is determined by the increased levels of catalase and superoxide dismutase activities. And in these flies which were treated with the Methotrexate along with the plant sample these enzyme activities are lower, there by indicating in the reduction in the stress levels.

MATERIALS AND METHODS

Fly rearing

The Drosophila Stock Centre, Department of Zoology University of Mysore, provided us the stocks of wild type *Drosophila melanogaster,* further the flies were cultured on the media made out of rava, jaggery and the files were maintained between 20-25 degrees.

Stress induction

Methotrexate abbreviated as MTX, formerly known as amethopterin, a antimetabolite and antifolate drug, it acts by inhibiting the metabolism of folic acid. This methotrexate was dissolved in alcohol and mixed along with the media in different concentration in the range of 5ppm, 10ppm, 15ppm, 20ppm, 25ppm, to the different flies groups.

Enzymatic assays of catalase and Superoxide dismutase (SOD)

Different set of flies were homogenized at cold condition and the homogenised mixture was centrifuged at 8000 rpm for 20 mins and the supernatant was taken as enzyme source.

Catalase activity assay

Catalase (EC 1.11.16) was assayed by Beers and Sizer (1952) method, for this the enzyme source of 0.1 ml and 2.9 ml of hydrogen peroxide is taken and the absorbance was measured by spectrophotometer at 270nm, and the protein estimation was done by Lowry's method and specific activity was calculated.

Superoxide dismutase assay (SOD)

Superoxide activity was determined by Beauchamp and Fridovich (1971). For this assay different cocktails of phosphate buffer,methionine, Riboflavin, NBT (Nitroblue tetrazolium) and the enzyme source was used and the test tubes containing this mixture was exposed to 400w bulb for 15 mins and the protein estimation was carried out by Lowry's method.

RESULTS

Reduction in the catalase activity

In flies which were exposed to Methotrexate, the stress inducing agent used in this study the catalase activity was higher but in the flies group which were

fed with methotrexate along with the plant sample the catalase activity was reduced .This reduction in the catalase activity decreases in a concentration dependent manner

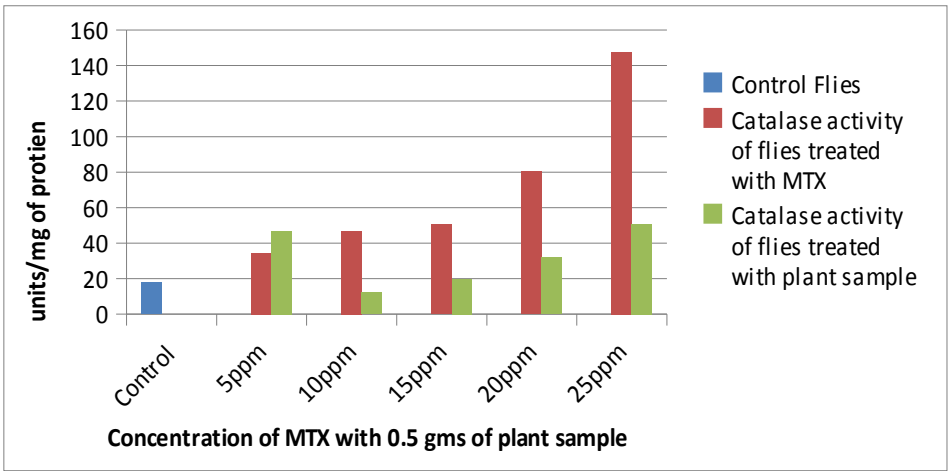

Figure 1. Specific activity of catalase enzyme in control flies, MTX (Methotrexate) induced flies and in flies treated with plant sample.

Superoxide dismutase (SOD) activity

In flies which were exposed to Methotrexate, the stress inducing agent used in this study the SOD activity was higher but in the flies groups which were fed with methotrexate along with the plant sample the SOD activity was reduced .This reduction in the catalase activity decreases in a concentration dependent manner.

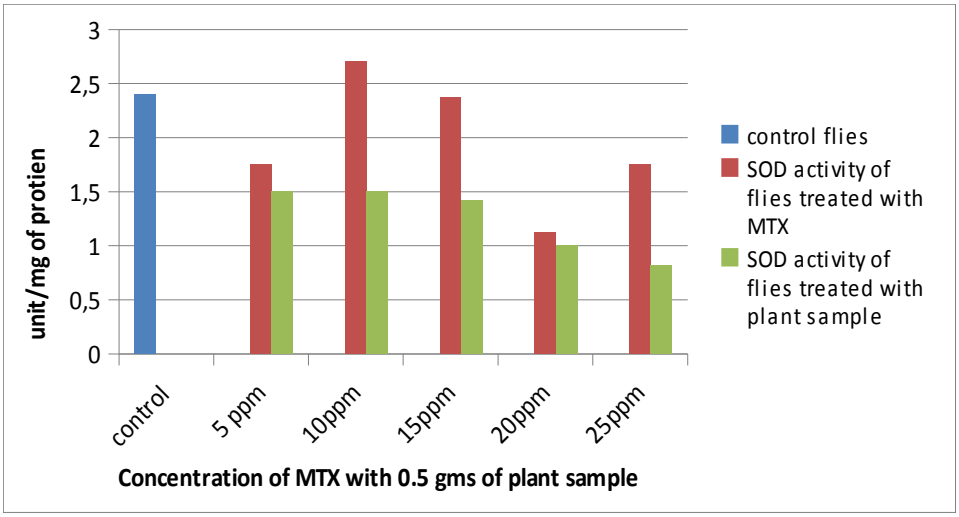

Figure 2 Specific activity of SOD enzyme in control flies, MTX (Methotrexate) induced flies and in flies treated with plant sample.

DISCUSSION

Stress and multiple stressors can lead to many problems such as autoimmune diseases. Oxidative stress is a condition characterized by elevated levels of intracellular reactive oxygen species (ROS). ROS either are, or break down to form, free radicals. ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) that are capable

of reacting with, and damaging DNA, proteins, and lipids.

It is suggested that oxidative damage to proteins plays a crucial role in ageing because oxidized proteins lose catalytic function and are preferentially hydrolyzed. It is hypothesized that oxidative damage to specific proteins constitutes one of the mechanisms linking oxidative stress/damage and age-associated losses in physiological functions (Sohal, 2002).

Mammalian cells possess elaborate defense mechanisms to detoxify radicals. The key metabolic steps are SOD catalysis of the dismutation of superoxide to hydrogen peroxide and oxygen and the conversion of H_2O_2 to O_2 + H_2O by catalase (Spranger et al., 1997).

The activity of Catalase and SOD increases significantly in a concentration dependent manner after inducing stress. One possible reason is that the stress inducing agent MTX may cause oxidative stress and much ROS is produced. In order to antagonize ROS, defensive enzymes such as Catalase and SOD activities are involved

As per our results, the plant powder was found to reduce the stress induced by the stress against Methotrexate in *D. melanogaster*.

The Catalase and SOD activity were increased in the Drosophila, which were grown in the media containing different concentrations of MTX. This was confirmed by comparing the activity of enzymes with that of the normal or control flies.

Simultaneously the activity of the enzymes were decreased in the stress induced flies, when they were reared on the media containing 0.5 gm of plant sample along with different concentrations of MTX.

Some of the flies were reared in the media, in the presence of only 0.5 gm. of plant sample. The Catalase activity in these flies was found to be decreased slightly, indicating that the plant sample may be effective in suppressing the Catalase activity .In SOD activity in this group of flies was found to be same as the control, indicating that the plant sample may be effective in suppressing the SOD activity in *Drosophila*.

Superoxide dismutases (SOD) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen.

SOD is also a part of the defense system against oxidative stress in aerobic organisms, it catalyses superoxide anion (O_2) and hydrogen peroxide, which then reduces to water (by hydrogen peroxide scavenging enzyme- Catalase).

Therefore Catalase and SOD are thought to limit the accumulation of reactive oxygen species.

The result of the present study showed that the plant powder may have the anti-stress property as it reduced the stress, which was demonstrated by the reduced activities of enzymes like SOD and Catalase in stress induced *Drosophila melanogaster*.

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