Neuroprotection and antioxidant evaluation of methanol extract of *Sida glutinosa* using *Drosophila* model system.

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Abstract

Oxidative stress is a harmful condition that occurs when there is an excess of free radicals or a decrease in antioxidant levels. The evidence to date for oxidative stress in Parkinson's disease (PD), Schizophrenia (SCZ), Alzheimer's disease (AD), and other neurodegenerative diseases is strongly persuasive. Clinical studies showed that a number of events associated with Alzheimer's are capable of stimulating production of free radicals and depletion of antioxidant levels. As pointed out, whether oxidative stress is eventually proven to be primary or secondary in etiologic progression, the therapeutic rewards of antioxidants are likely to be substantial. Clearly, strategies aimed at limiting free radical induced oxidative stress and damage may slow the progression of neurodegenerative diseases (Ravindra *et al*., 2004).

Paraquat (1,1-dimethyl-4,4-bipyridynium dichloride) is a quaternary nitrogen herbicide and highly toxic substance for humans and animals; many cases of acute poisoning and death have been reported (Sittipunt, 2005). The toxicity of paraquat is due to the generation of the superoxide anion, which can lead to the synthesis of more toxic reactive oxygen species (ROS) such as hydroxyl radicals and hydrogen peroxide (Suntres, 2002). On the other hand, the oxidation of reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) as a consequence of paraquat administration results in the disruption of biochemical processes requiring NADPH (Smith *et al*., 1978).

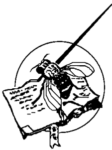
The use of antioxidant compounds provides an easy and convenient way of testing the validity of the free radical theory using *Drosophila melanogaster* as model organism. The organisms are easier to culture and manipulate under laboratory conditions than the mammals. Administration of the test compounds can be done easily by adding the dissolved compounds to the food medium thus ensuring their uptake by the flies (Tapiwanashe *et al*., 2006).

Sida glutinosa plant belongs to family *Malvaceae*, commonly called as "*Sticky Fanpetals*". It is a sub-shrub which is available in the forest at shady areas along Ravines. It was identified in Biligiri Rangana Hills of Chamarajanagar District, Karnataka, India.

In this paper methanol extract of *Sida glutinosa* (**G**) was preliminary screened for Physico-chemical, Phyto-chemical properties, *in vitro*, *in vivo* antioxidant and neuroprotective studies were carried out using *Drosophila melanogaster* (Oregon K) strain adult male flies. *In vitro* antioxidant studies exposed that the extract exhibited concentration dependent activity. The oxidative stress markers employed in the study included lipid peroxidation products malondialdehyde (MDA) and hydroperoxide (HP), reduced glutathione (GSH), and antioxidant enzymes included superoxide dismutase (SOD) and catalase (CAT). The oxidative stress was induced by using paraquat at 15 mM. The concentration of extract for studies was fixed based on LC50 values. There was significant

demolition in the levels of MDA and HP in case co exposure of **G** with Par treated flies homogenate. The level of SOD and CAT were brought to near basal level in the homogenate of flies co exposed with **G** and Par. In negative geotaxis assay it was found that **G** was able to rescue the flies significantly from deteriorating locomotors dysfunctions. The extract **G** showed significant antibacterial property against tested strains.

References: Ravindra, P.S., S. Shashwat, and K. Suman 2004, JIACM 5: 218-225; Sittipunt, C., 2005, Respir. Care 50: 383-385; Suntres, Z.E, 2002, Toxicol. 180: 65-77; Smith, L., M.S. Rose, and I. Wyatt 1978, Ciba Found. Symp. 65: 321-341; Tapiwanashe, M, W. Melanie, R. Kumars, P.M. Michael, A.J. Robin, and L.P. Smith 2006, Mech. Age. Devpt. 127: 356-370.



Sequence variations in dosage compensation genes and histone deacetylases in *In(1)B^{M2}(reinverted)* of *Drosophila melanogaster.*

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The *Drosophila* strain *In(1)B^{M2}(reinverted)* arose in 1978 as a spontaneous re-inversion in *In(1)B^{M2}* (inversion break points 16A-20F) (Mazumdar *et al*., 1978). This re-inverted strain manifests a sex and chromosome specific alteration of the male X chromosome, which can either be induced through continuous rearing at 18 ± 1°C (Mukherjee and Ghosh, 1986) or through a brief exposure to cold shock at 12 ± 1°C for four hours (Kar *et al.,* 2000). The alteration in the structure of the male X chromosome is associated with a transient, male specific increase in the acetylation of histone H4 at lysine 16 (H4K16) (Kulkarni-Shukla *et al.,* 2008). This male specific acetylation is brought about by the gene *males absent on the first* (*mof*), a histone acetyltransferase that is a component of the multiprotein complex that brings about dosage compensation in *Drosophila melangoster* (Bone *et al*., 1994). Due to the male sex specific phenotype and the observed hyperacetylation, we investigated whether the structural alteration of the male sex chromosome occurred due to mutations in the genes of the dosage compensation pathway (*i.e*., *mof*, *male- specific lethal 1*, *msl-1*; *male-specific lethal 2, msl-2; male-specific lethal 3*, *msl-3*; *maleless, mle*; *RNA on the X 1, roX1;* and *RNA on the X 2, roX2*) chromatin remodelling genes associated with dosage compensation (*Imitation SWI*, *Iswi*; *Trithorax-like*, *Trl*; *supercoiling factor, scf*; and *JIL-1*) or due to mutations perturbing the activity of histone deacetylases (HDAC) (*Rpd3, HDAC6, Histone deacetylase 3* [*Hdac3*], *HDAC4,* and *Sir2*).

The sequences of these sixteen genes were identified using FlyBase (Tweedie *et al.,* 2009), and primers were designed using Primer3 primer design programme (Rozen and Skaletsky, 2000). Amplifications and sequencing were carried out in triplicates. The gene sequence variations were determined by aligning them to the wild type sequences obtained from FlyBase using ClustalW2 with default settings (Larkin *et al.,* 2007). Coding sequences were translated using EMBOSS Transeq (http://www.ebi.ac.uk/Tools/ emboss/transeq / index), and the predicted protein sequences for each gene were aligned to that of the wild type using ClustalW. Nucleotide variations were categorized as synonymous, non-synonymous, conserved or semi conserved depending on the resultant amino acid change.

Of the sixteen genes only one, HDAC, *Rpd3* showed 100% homology to the wild type sequence reported in the FlyBase. Table 1 shows the variations reported for the other fifteen genes.