

## Effect of *Bacopa monniera* (Linn.) on lipid peroxidation and lipofuscinogenesis in prostate gland of D-galactose induced aging mice, *Mus musculus*

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The levels of malondialdehyde and lipofuscin pigments were increased in the prostate of D-galactose (0.5 ml/day, sc, for 20 days) induced aging mice. After *B. monniera* (40 mg/kg body weight for 20 days) ethanol leaf extract administration levels of both the parameters were reduced significantly. The results suggest that *B. monniera* prevents formation of malondialdehyde and lipofuscin pigments which are the indicators of aging.

**Keywords:** Antioxidants, *Bacopa monniera*, D-galactose, Free radicals, Lipid peroxidation, Lipofuscin pigments

Increasing levels of free radicals and decline in natural antioxidant system of the body during aging are responsible for pathogenesis of prostate diseases<sup>1</sup>. Dietary intake of antioxidants prevent the prostatic disorders. Men taking vitamin E, a potent antioxidant are far less likely to develop prostate cancer than the men who don't<sup>2</sup>. Lycopene, one of the carotenoid pigment in tomatoes, is a well-known antioxidant which exerts its chemopreventive effects by modulating the oxidant-antioxidant profile in the target organ<sup>3</sup>. Diets rich in tomatoes appear to confer some protection against prostate cancer showing that failure in natural antioxidant system is responsible for the prostate diseases<sup>4</sup>. Vitamin E and carotenoid<sup>5</sup> scavenge free radicals. Vitamin E prevents oxidation of polyunsaturated fatty acids of the membrane and protects the cell membrane integrity which is necessary for normal cellular and organelle functions<sup>6</sup>. Vitamin E deficiency is associated with the accumulation of lipofuscin deposits which is

indicative of free radical induced oxidation of membranous organelles<sup>5</sup>. Preventive effects of vitamin C were observed on lipid peroxidation in aging rats<sup>7</sup>. Selenium has beneficial effects in reducing the occurrence of prostate cancer<sup>8</sup>. Coudray *et al.*<sup>9</sup> found that selenium and antioxidant vitamins reduce the lipid peroxidation level in pre-aging French population. Thus, intake of antioxidants protects the prostate from old age defects like lipid peroxidation and other defects and may prevent pathological disorders of prostate.

Tripathi *et al.*<sup>10</sup> showed antioxidant effect of ethanolic extract of *Bacopa monniera* against ferrous sulphate cumen hydroperoxide induced lipid peroxidation in rat liver. *B. monniera* has dose dependent free radical scavenging capacity and protective effect on DNA damage<sup>11</sup>. Many therapeutic uses of *Bacopa* have been described in traditional medicine. In Ayurvedic system of medicine it is used as a brain tonic to promote mental health and improve memory and intellect. Anxiolytic, relaxing, bronchodilatory, cognition enhancing, immunomodulating and anti-inflammatory properties of *Bacopa* are reported. *Bacopa* contains different types of steroid saponins, notably Bacoside A and B, which facilitate the capacity of mental retention in rats<sup>12</sup>.

The above literature indicates that *Bacopa* has rich antioxidant properties and can prevent lipid peroxidation and lipofuscinogenesis, which are the markers of aging, hence effect of *Bacopa* have been studied on the above parameters in the aging prostate in rats.

Leaves of *Bacopa monniera* (Linn.) were collected from Town Hall Garden, Kolhapur. The plant was identified by Taxonomist from Botany Department, Shivaji University, Kolhapur where a voucher specimen (Exsiccata: MMS 1956) has been preserved. The leaves were air dried. Ethanol leaf-extract of *Bacopa* was prepared as per Tripathi *et al.*<sup>10</sup>. Five months old male mice (*Mus musculus*) of Swiss albino strain, weighing about 40 g, were maintained in the plastic cages in AC animal house (CPCSEA No.233) under 12:12 hr L:D cycles. The animals were provided with pelleted food from 'Pranav Amrut food', Kolhapur and water *ad libitum*. The study protocol was approved by the institutional Animal Ethical Committee. Care of animals was taken as per

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guidelines of CPCSEA, Dept. of Animal Welfare Govt. of India.

The mice (25) were divided into following 5 groups of 5 animals each:

Control Group I: Animals were injected subcutaneously (sc) with 0.5 ml sterile water/day/animal for 20 days.

Aging induced Group II: The mice were injected (sc) with 5% D galactose, 0.5 ml/day/animal for 20 days to induce aging. Deshmukh *et al.*<sup>13</sup> have confirmed the dose and duration of D-galactose for induction of aging in mice.

*Bacopa* co-treated Group III: The mice were injected (sc) with 5% D-galactose along with ethanol extract of *Bacopa* leaves (40 mg/kg body weight), 0.5 ml/day/animal. The co-treatment of *Bacopa* extract was carried out to study its effect on induced aging and oxidative stress.

Natural recovery Group IV: This group was injected with 0.5 ml of 5% D-galactose, for 20 days and then they were kept without any dose of *Bacopa* for next 20 days to study the natural recovery, if any.

*Bacopa* recovery Group V: The mice were injected with 0.5 ml 5% D-galactose for 20 days and then were injected with ethanol extract of *Bacopa* leaves (40 mg/kg body weight) for next 20 days, to study effect of *Bacopa* as antiaging agent and to study the recovery by *Bacopa*.

On completion of doses the animals were sacrificed by cervical dislocation; prostate gland was dissected out, blotted and weighed. The prostate tissue was homogenized by using mixture containing 75 mM phosphate buffer (pH 7.04), 1 mM ascorbic acid and 1 mM ferric chloride.

Lipid peroxidation was studied by Will's<sup>14</sup> method in which the thiobarbituric acid reactive substance (TBARS) i.e. malondialdehyde (MDA) is measured in the form of red coloured malondialdehyde-TBA complex colorimetrically at 532 nm using standard. Lipid peroxidation was measured in the form of n mol MDA/mg wet weight of tissue.

Lipofuscinogenesis was studied by the method of Dillard and Tappel<sup>15</sup>. The prostate tissue was

homogenized by using the mixture prepared earlier for lipid peroxidation. The extraction was carried out by addition of chloroform:methanol (2:1 v/v) to 0.5 ml of homogenized tissue sample. It was mixed well on vortex mixer and then 3ml of double distilled water was added and centrifuged at 300 g for 2 min. To 1 ml of upper layer 0.1 ml of methanol was added and the fluorescence was measured on spectrofluorometer (Elico, SL, 174) at 366 excitation spectrum and 450 emission spectrum.

All values are expressed as mean  $\pm$  SD. The statistical analysis was performed using oneway Analysis of variance (ANOVA) followed by Tukey's Post Hoc Test. A value of  $P < 0.01$  was considered statistically significant.

Lipid peroxidation, which indicates endogenous MDA levels, was found to be increased in the prostate of D-galactose treated mice as compared to age matched control mice (Table 1). On the other hand it decreased in *Bacopa* co-treated group as compared to D-galactose induced mice. In natural recovery group MDA concentration increased as compared to the *Bacopa* recovery group. Intensity of fluorescence indicated that lipofuscinogenesis was increased in aging induced mice than the control, where as it was reduced due to *Bacopa* co-treatment. The decrease in lipofuscins in *Bacopa* recovery group was higher than the natural recovery group.

In the present investigation D-galactose was used to induce aging. D-galactose is a reducing sugar that can form advanced glycation end product (AGE) *in vivo*<sup>16</sup>. The elevated AGEs may accelerate the aging process because AGEs are responsible for free radical production, particularly superoxide radicals, by oxidation and degradation of glycated proteins. They induce the intracellular oxidative stress through AGE- receptor mediated activation of signaling pathways and thus induce the respiratory burst<sup>17</sup>. In their study Song *et al.*<sup>16</sup> found that both D-galactose and AGE treated mice resemble naturally aged mice which suggests that AGE also accounts for inducing aging.

Table 1 — Effect of *B. monniera* on lipid peroxidation and intensity of fluorescence in D-galactose induced mice.

	[Values are mean $\pm$ SD from 5 animals in each group]				
	Control	Gr. II	Gr. III	Gr. IV	Gr. V
MDA (n Mol /mg tissue)	1.28 $\pm$ 0.07	1.60 $\pm$ 0.08	1.12 $\pm$ 0.06	1.44 $\pm$ 0.07	1.04 $\pm$ 0.05
Intensity of fluorescence	87 $\pm$ 1.0	125 $\pm$ 1.2	97.5 $\pm$ 0.8	84.7 $\pm$ 1.1	41.3 $\pm$ 0.7

Gr.II: D-galactose induced aging, Gr.III: *Bacopa*-co-treatment, Gr.IV Natural recovery group, Gr.V: *Bacopa* Recovery  
ANOVA followed by Tukey's test,  $P < 0.01$

In the present study after treatment of D-galactose there was increase in the level of MDA and lipofuscinogenesis in the mice prostate, which was probably because of generation of AGE<sup>16</sup>, but co-treatment of Bacopa reduce levels of both indicating preventive effect of Bacopa against the action of D-galactose. Bhattacharya<sup>18</sup> had shown that oral administration of ethanolic extract of *Bacopa* in rat resulted in the increase in antioxidant enzymes like superoxide dismutase, catalase and glutathion peroxidase in frontal cortex and hippocampus. These are the naturally occurring antioxidant enzymes which scavenge the free radicals. Bacopa contains different types of steroid saponins, notably bacosides A and B, which were found to facilitate the capacity of mental retention in rats<sup>12</sup>. In the present work, in case of the prostate of natural recovery group both lipid peroxidation and lipofuscin levels were more as compared to *Bacopa* recovery group, indicating repairing effect of *Bacopa*. Whatever damage due to D-galactose treatment was done in the prostate was found reduced after *Bacopa* treatment but the damage was retained in the prostate of natural recovery group. The antioxidant effect of *Bacopa* may help to reduce AGE formation like other AGE inhibitors<sup>19</sup> and hence the aging effects in the prostate.

In conclusion the present study indicates that imbalance between oxidants and antioxidants during aging can be minimized by *Bacopa monniera* which may be beneficial in reducing the aging effects in case of the prostate.

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