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Original Article

EFFECT OF BACOSIDE A ON LIPID PEROXIDATION IN D-GALACTOSE INDUCED AGING MICE

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ABSTRACT

Objective: Bacoside A is a major bioactive constituent of *Bacopa monnieri* L having antioxidant property. The objective of this study was to evaluate the effect of Bacoside A, on lipid peroxidation in brain, heart and liver during induced aging.

Methods: Male Swiss albino mice, *Mus musculus* was used for the present investigation. Four experimental groups were used as Group I-Normal adult, Group II-D-galactose induced, Group III - D-galactose induced plus Bacoside A treated and Group IV - Natural aging. The effect of Bacoside A was studied against lipid peroxidation during induced aging. The level of lipid peroxidation in the form of MDA formation was determined and measured in brain, heart and liver.

Results: The statistical data obtained were analyzed using one way ANOVA, control vs other groups and results were expressed as mean±SE. In Bacoside A treated group the lipid peroxidation level in heart, brain and liver was significantly decreased ($p<0.001$) compared to control group. A significant increase ($p<0.0001$) in the level of lipid peroxidation was observed in D-galactose induced mice. In natural aging group highly significant increase ($p<0.0001$) in initial lipid peroxidation, ascorbate dependent lipid peroxidation and spontaneous lipid peroxidation was observed.

Conclusion: The observations revealed that, lipid peroxidation was reversed in Bacoside A treated group which may be due to antioxidant property of Bacoside A. Thus Bacoside A is able to ameliorate the stress induced changes in lipid peroxidation during aging. The findings also provide a theoretical basis for the development of novel therapeutic formulations, such as antioxidant supplementation to boost antioxidant defenses in the body.

Keywords: Oxidative stress, Aging, Lipid peroxidation, D-galactose, Bacoside A, Antioxidant

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INTRODUCTION

Lipid peroxidation is the product of chemical damage done to the lipid component of cell membranes by oxygen free radicals [1-3]. This damage is thought to be a basic mechanism underlying many diverse disorders such as atherosclerosis, cancer, aging, rheumatic diseases, cardiac and cerebral ischemia, various liver problems and toxicity induced by harmful environmental factors. It has been considered that the lipid peroxidation damage is involved in aging and pathological disorders [4, 5]. Reactive Oxygen Species (ROS) are constantly generated in living cells as a part of intracellular metabolic processes and induce oxidative damage to cell membranes, lipids, proteins and nucleic acids [6, 7]. The free radicals that contribute to the aging process are derived directly or indirectly from oxygen. As lipid peroxidation traditionally has been regarded as the major process that produces damage to oxygen radicals and the oxidized lipid residues are major components of lipofuscin, the fluorescent pigment that accumulates with age in most tissues [8-10]. Post mitotic cells are very susceptible to the oxidative damage due to their high consumption of oxygen and presence of fatty acids that are prone to peroxidation. Thus damage due to lipid peroxidation is evident in a wide range of degenerative diseases and aging [6, 11]. A reducing sugar, D-galactose reacts with free amino groups of amino acids and proteins to form insoluble agglomerates called as advanced glycation end products, AGE's [12, 13]. The advanced glycation end products formed due to D-galactose accumulates in cells and provokes the formation of free radicals which are responsible for the pathogenesis of various diseases and aging as well [14].

Many researchers have focused on natural antioxidants, numerous crude extracts and pure natural compounds present in plants. A large amount of secondary metabolites are present in medicinal plants. Flavonoids, saponins and polyphenols in plants are a versatile group of antioxidants that protect against damage caused due to lipid peroxidation by directly neutralizing reactive oxygen species [15-22]. It is therefore important to study the effect of secondary metabolites on a particular tissue. *Bacopa monnieri* (L) traditionally used to treat various human ailments. It has been reported to have possible medicinal attributes as an antioxidative, antimicrobial, anti-aging and free radical scavenging activity [23-28]. Triterpenoid saponins, the major components in Brahmi, were reported to be responsible for the cognitive enhancing activity of Brahmi [24, 26, 29]. The major bioactive dammarene type triterpenoid saponin isolated from the *Bacopa monnieri* (L), that carries the neuropharmacological activities, is Bacoside A [30]. In this context, the study has been carried out to evaluate the effect of Bacoside A on lipid peroxidation in D-galactose induced aging mice.

MATERIALS AND METHODS

Chemicals and reagents

D Galactose, Potassium chloride (KCl), Ascorbic acid, Ammonium ferrous sulphate (Mohr's salt), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA) were purchased from LOBA chemie Pvt. Ltd., Mumbai, India.

Animals

Male mice (*Mus musculus*) of Swiss albino strain of different age groups were reared in animal house. Approval was obtained by the CPCSEA and IAEC (CPCSEA/PCL/23/2014-15). All instructions and rules of CPCSEA were followed. Animals were kept under a 12:12 hr L: D cycle and fed *ad libitum* a commercial diet provided by Pranav Agro Industries, Pune. Animals were divided into the following four experimental groups, six animals each group:

Group I-Normal adult (control) (12 Mo Age)

Group II-D-galactose induced (12 Mo Age)-injected with D-galactose subcutaneously for 56 d

Group III - D-galactose induced+Bacoside A treated (12 Mo Age)- injected with D-galactose subcutaneously and administered Bacoside A orally at a dose of mg/kg body weight for 56 d

Group IV - Natural aging (18-20 Mo Age).

Plant

The Bacoside A ($C_{41}H_{68}O_{13}$) was purchased from M/S Natural Remedies Pvt. Ltd. Banglore, India.

Preparation of homogenate

The experimental animals were sacrificed after completion of doses. The brain, heart and liver were dissected out. The brain tissues were separated as cerebral hemisphere and cerebellum. The homogenates were prepared by using 0.9% KCL and centrifuged at 3000 rpm at 10 °C. The obtained homogenates were used for further study.

Lipid peroxidation assay

The rate of lipid peroxidation was measured by the method of Strove and Makarova 1980. The level of lipid peroxidation in the form of MDA formation was determined and measured in brain regions, heart and liver at 532 nm.

Statistical analysis

The results obtained were analyzed by the SPSS software package version 20. The mean values obtained for the different groups were compared by one-way ANOVA, followed by Dunnett test, Normal adult (control) vs other groups. The results were expressed as mean±SE and P<0.001 was considered as highly significant.

RESULTS AND DISCUSSION

In the present study, the level of lipid peroxidation in brain, heart and liver of experimental groups was measured. The lipid peroxidation in the form of MDA formation in cerebral hemisphere, cerebellum, heart and liver were depicted in table 1 and Graph No. 1,2,3,4. In Bacoside A treated group the lipid peroxidation level in heart, brain and liver was significantly decreased (p<0.001) which some extent resembles as in the normal adult. A significant increase (p<0.0001) in the level of lipid peroxidation was observed in D-galactose induced mice. In natural aging group highly significant increase (p<0.0001) in initial lipid peroxidation, ascorbate dependent lipid peroxidation and spontaneous lipid peroxidation was observed.

In vivo lipid peroxidation has been identified as a basic deteriorative reaction in cellular mechanisms of aging processes [5, 31]. As highly reactive free radicals are not removed from the cell, the propagation of free radical reaction may be increasing exponentially in old age. The pattern of damage to proteins induced by peroxidizing lipid is similar to radiation damage [32]. Effect of age on lipid peroxidation in various parts of the rat brain was studied by Koudelova and Mourk, a significant increase in MDA production in cerebral cortex was observed [33]. Age related changes in lipid peroxidation were observed by Pawar, the lipid peroxidation was increased in old male mice than in adult male mice [34]. It was reported that feeding of *Murraya koenigii* and *Brassica Juncea* decreased the level of lipid peroxidation in liver and heart of rats [15]. The significant reduction in the level of lipid peroxidation was observed in rats liver and heart treated with methanol extract of *Teramnus labialis* [19].

When the level of D-galactose increases above the normal, it gets oxidized into hydrogen peroxide and aldehydes [35]. Increased level of it induces premature aging due to increased advanced glycation end products, decreases motor activity and stimulates diabetes [36]. It has been reported that D-galactose responsible to deflate immune responses, accelerate oxidative stress by increasing lipid peroxidation and decline antioxidant enzyme activities by prevailing degeneration [37]. The study on aging showed that in D-galactose treated mice increased levels of lipid peroxidation indicates ageing associated changes since during aging there is increased production of reactive oxygen species, hence increased lipid peroxidation [38]. It was observed that the animals administered *Petroselinum crispum* extract along with D-galactose showed the significantly low level of MDA in the brain as compared to the D-galactose treated mice [11]. Increased malondialdehyde is an indication of increased lipid peroxidation in D-galactose treated mice that results due to increased oxidative stress [39, 40].

The results from our study revealed that the concentration of MDA in brain regions, heart and liver of D-galactose treated group was elevated as compared to control group. In the animals which received Bacoside A along with D-galactose, MDA level was significantly less in brain regions, heart and liver as compared to D-galactose treated group. The results show that administration of Bacoside A brings about alterations in the level of lipid peroxidation in different tissues. The concentration of MDA decreased significantly in the brain, heart and liver of Bacoside A treated group hence Bacoside A has ameliorative effects on D-galactose induced mice. The level of lipid peroxides in Bacoside A administered group suggests that it can able to revert the effects caused due to D-galactose and can maintain the concentration of MDA at the normal level during induced ageing.

Table 1: Formation of TBA reacting products in spontaneous, ascorbate dependent and initial lipid peroxidation (n mols malondialdehyde/mg tissue/1h.) in cerebral hemisphere, cerebellum, heart and liver

Organ	Cerebral hemisphere			Cerebellum			Heart			Liver		
group	X1	X2	X3	X1	X2	X3	X1	X2	X3	X1	X2	X3
Normal Adult	39.20 ±0.1006	9.502 ±0.0213	0.8263 ±0.0075	45.68 ±0.0138	6.678 ±0.0060	1.063 ±0.0245	31.49 ±0.1407	4.770 ±0.0057	0.6728 ±0.0014	41.13 ±0.2493	5.832 ±0.0144	1.345 ±0.0585
D-galactose induced	43.92 ±0.0482	11.65 ±0.0135	1.513 ±0.0016	48.30 ±0.0338	14.87 ±0.0100	1.873 ±0.0024	39.95 ±0.0135	5.125 ±0.0120	1.013 ±0.0010	46.98 ±0.0076	9.055 ±0.0088	2.067 ±0.0007
D-galactose+Bacoside A treated	39.77 ±0.1545	6.147 ±0.0114	0.5380 ±0.0006	42.12 ±0.0788	11.67 ±0.0114	0.8865 ±0.0050	29.33 ±0.0130	4.858 ±0.0600	1.090 ±0.0025	31.65 ±0.1174	7.237 ±0.0311	1.649 ±0.0017
Natural aging	49.78 ±0.0065	14.29 ±0.0061	1.816 ±0.1239	52.90 ±0.0057	17.07 ±0.0202	2.328 ±0.0543	48.45 ±0.1869	6.702 ±0.1269	1.519 ±0.0386	50.47 ±0.0589	11.38 ±0.1216	3.169 ±0.0040

All values of D-galactose, induced, D-galactose+Bacoside A treated group and Natural aging group are compared with respect to the normal adult (control) group, Values are expressed as mean±SE (n=6 mice), X1-The rate of spontaneous lipid peroxidation in the homogenates nmoles of malondialdehyde formation during 1h. X2-The rate of ascorbate dependent non-enzymatic peroxidation in the homogenates nmoles of malondialdehyde formation during 1h. X3-The amount of malondialdehyde in the initial homogenate

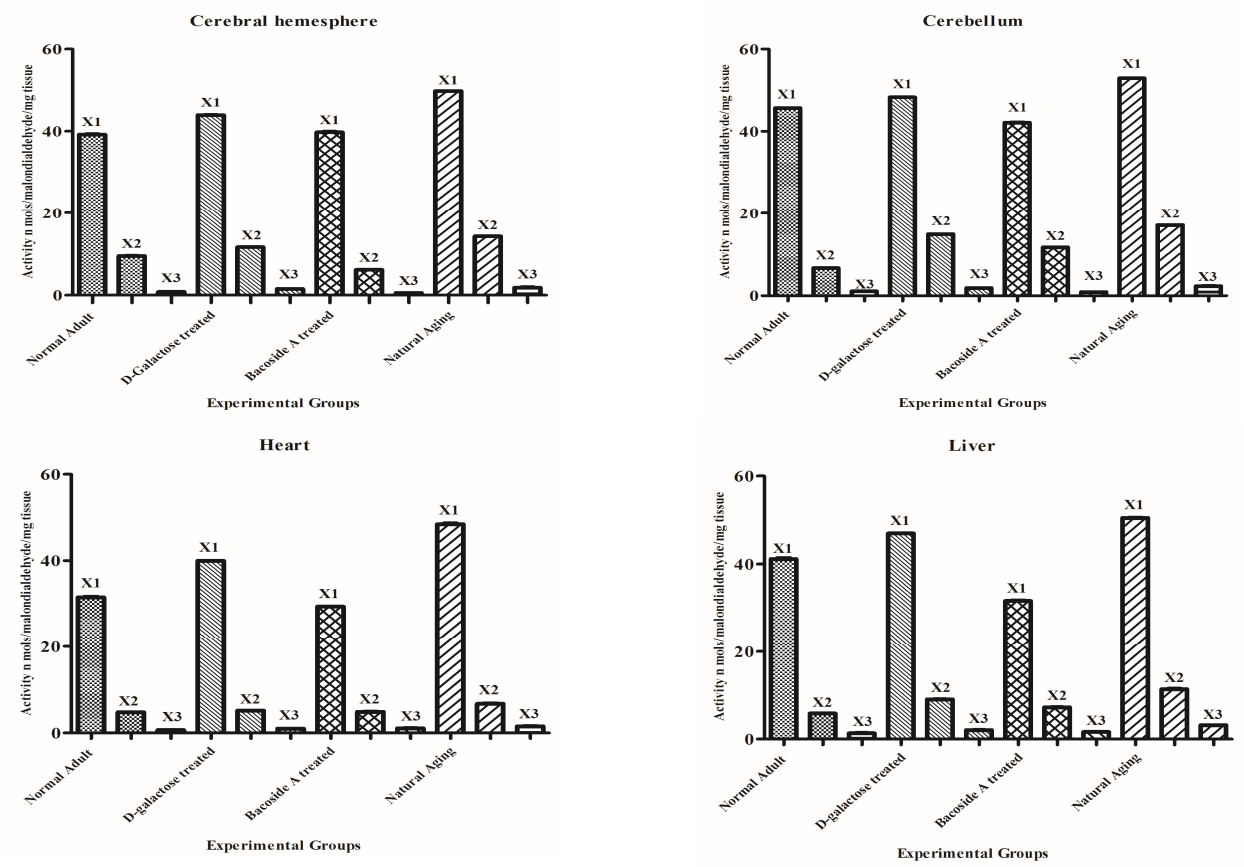

Fig. 1: Formation of TBA reacting products in spontaneous, ascorbate dependent and initial lipid peroxidation (n mols malondialdehyde/mg tissue/1h.) in Cerebral hemisphere, cerebellum, heart and liver

CONCLUSION

The present study concluded that increased level of lipid peroxidation may be due to formation advanced glycation end products by D-galactose. Maximum protection against lipid peroxidation damage can be achieved by using sufficient concentrations of natural antioxidants. Thus, the present results suggest that Bacoside A may have the potential to reduce the formation of lipid peroxidation during induced aging.

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AUTHORS CONTRIBUTION

Authors are equally contributed in this work**.**

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest

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