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Effect of *Terminalia chebula* on monoamine oxidase and antioxidant enzyme activities in aged rat brain

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ABSTRACT - The aging presents itself with various alterations in physiological events. The free radical theory of aging reflects the free radical induced damage to cellular components and increases in catecholamine metabolism in aging. Activities of MAO-B, MDA, MnSOD, CAT were showed a significant increase and decrease in the activity of GPx in brain mitochondria of aged rats as compared to young control rats. Administration of *Terminalia chebula* aqueous extract 200mg/kg body weight orally for 4 weeks to aged rats showed a marked decrease in the activities of brain MAO-B, MDA, MnSOD, CAT activities and significant increase in the activity of GPx. *T.chebula* could prevent MAO-B induced oxidation and lipid peroxidation in the brain of aged rats. Thus the present study revealed that the antioxidant potential of *T. chebula* by protecting the brain cells against free radical toxicity through the inhibitory action on catecholamine oxidation and lipid peroxidation in aged rats and modulating antioxidant defense systems.

KEYWORDS - Aging, antioxidants, lipid peroxidation, monoamine oxidase-B, *Terminalia chebula*

INTRODUCTION

Many biochemical processes and physiologic functions are impaired with normal aging and thereby resulting in a number of detrimental health consequences in the elderly populations (1). The free radical theory of aging predicts that the rate of aging is dependent on the level of oxidative stress, i.e., the balance between pro-oxidants and antioxidants and the consequent oxidative damage (2). A prominent feature that accompanies aging is an increase in brain mitochondrial monoamine oxidase (MAO, EC 1.4.3.4) levels. MAO-B is responsible for the metabolism of biologically important active amines and oxidative deamination of these amine produces NH_3 and H_2O_2 with established or potential toxicity to mitochondria (3,4). Cells are equipped with an impressive repertoire of antioxidant enzymes as well as radical scavengers. They are manganese superoxide dismutase (MnSOD), catalase (CAT) and glutathione peroxidase (GPx), scavenging H_2O_2, generated either during the dismutation of superoxide anion radicals, or in the process of catecholamine deamination by MAO (5).

T. chebula (Combretaceae) (Chebulic myrobalan in English) is a native plant in India and used in Indian system of medicines such as Ayurveda and Siddha for treating liver diseases, urinary disorders, and heart diseases, ulcer, diabetes, arthritis, neuropathy, memory loss, etc. (6). It is a well-known ayurvedic rasayana and also possess adaptogenic property (7). *T. chebula* is one of the ingredients in popular ayurvedic formulation of Triphala (8). The important active principle constituents of *T. chebula* are chebulagic, chebulinic acid, corilagin (9), beta-sitosterol, gallic acid, terchebulin, caffeic acids, carbohydrates, etc. (10). It is highly nutritious and could be an important source of dietary supplement in vitamin C, energy, protein, amino acids and mineral nutrients (11). Pharmacological actions of *T. chebula* indicate that cardioprotective (12), antioxidant activity (13), anticancer (14), antidiabetes (15), antimutagenic (16) and hypolipidemic (17). The present study was undertaken to examine the antioxidative potential role of *T. chebula* in brain of aged rats as compared with young rats.

MATERIALS AND METHODS

Chemicals

2-thiobarbituric acid, glutathione (reduced) were obtained from Sigma (St. Louis, MO, USA). All other chemicals were analytical grade marketed by Sisco Research Laboratories Pvt. Ltd., Mumbai and Glaxo Fine Chemicals Ltd., Mumbai, India.

Preparation of T. chebula aqueous extract

The fruits of *T. chebula* ripen from November to March and fall soon after ripening. The fully ripe fruits were collected from Kolli hills, Tamilnadu, India during the

month of January 2005 from the ground as soon as they have fallen and shade dried. Hundred grams of dried fruit skins were hammered in to small pieces followed by extraction with 800 ml distilled water for 24 h in water bath at 40°C and repeated for two times. The final yield of the aqueous extract 47.6% was noted and used for treatment of experimental rats.

Animals

Young (3-4 months, 120-150 g) and aged (22-24 months, 380-410 g) male albino Wistar rats were used for the experiments. The rats were housed in polypropylene cages on a 12L:12D cycle and fed *ad libitum* on commercial laboratory food pellets and water. All animal experiments were conducted as per the instructions of Institutional Animal Ethics Committee.

Experimental Design

The animals were divided into four groups of six each as namely, Group I **:** Control young rats were received sterile water only. Group II **:** Young rats were treated orally with *T. chebula* aqueous extract at a dose of 200mg/kg body weight in 1.5ml sterile water orally for 4 weeks. Group III **:** Control aged rats were received sterile water only. Group IV **:** Aged rats were treated orally with *T. chebula* aqueous extract as a dose of 200mg/kg body weight in 1.5ml sterile water orally for 4 weeks.

Preparation of brain mitochondria

On completion of 4 weeks of the experimental period, animals were anaesthetized with Thiopentone sodium (50mg/kg). Whole brain was excised immediately and immersed in physiological saline. Mitochondria in the brain were isolated using the method described by Satav and Katyare (18). Tissues were homogenized in the buffer containing 0.3 M mannitol, 0.1mM EDTA, pH 7.4. Homogenates were centrifuged at 600×g for 10 min at 4°C. The supernatant fractions were collected and followed by centrifugation at 10,000×g for 10 min 4 °C to obtain the brain mitochondria. The mitochondrial pellets were washed three times with 0.25 M sucrose buffer containing 0.1mM EDTA, pH 7.4, resuspended in 0.25 M sucrose buffer, pH 7.4 and stored at –80°C for further analyses.

Assay of monoamine oxidase-B (MAO-B)

The enzyme activity was estimated by the method of Green and Haughton (19) and Turski et al (20). The standard reaction mixture for MAO-B assay contained 0.025M phosphate buffer pH 7, 0.0125 M semicarbazide, 0.018M benzylamine (pH adjusted to 7) and enzyme equivalent to 3 mg protein in a total assay volume of 2 ml. Incubations was performed at 25°C for 30 min. After, 1ml of acetic acid was added and boiled for 3min in boiling water bath and then centrifuged. 1ml of supernatant was mixed with 1ml of 0.05% of 2,4-DNPH and 2.5ml of benzene was added after 10min incubation at room temperature. Then benzene layer was separated and mixed equal with 0.1N NaOH solution. Alkaline layer was decanted and heated at 80°C for 10min. The orange-yellow colour was read at 450nm. The enzyme activity was expressed as μmoles benzaldehyde semicarbazine formed/hour/3mg protein.

Assay of oxidation products and antioxidants

Lipid peroxidation was assessed biochemically by determining the level of malondialdehyde (MDA); the results were expressed as nmoles of MDA formed/mg protein using 1,1,3,3-tetraethoxypropane as standard (21). MnSOD activity was measured by the method of Kakker et al based on the combination of NADH-PMS-NBT (22), and results are expressed as 50% reduction of NBT/min/mg protein. Catalase (CAT) activity was measured according to the method of Beers and Sizer (23) in which disappearance of peroxide was followed spectrophotometrically at 240 nm; one unit of activity is equal to the μmol of H_2O_2 degraded/min/mg protein. Glutathione peroxidase (GPx) was estimated by the method of Rotruck et al (24) using reduced glutathione as substrate and results were expressed as μmole GSH utilized/min/mg protein. Protein concentrations were assayed by the method of Lowry (25) using bovine serum albumin as standard.

Statistical analysis

The values are expressed as mean ± standard deviation (SD). The results were computed statistically (SPSS software package) using one-way analysis of variance. Post hoc testing was performed for intergroup comparisons using the least significance (LSD) test. A *P*-value < 0.05 was considered significant.

RESULTS

In table 1, the levels of brain MAO-B and MDA were found significantly increased 65.03% and 27.72% respectively in aged control rats compared to young control rats. *T. chebula* administration decreased the levels of MAO-B and MDA significantly to 53.76% and 18.36% ($P<0.001$) with comparison of age-matched controls. Drug treated young rats also showed 13.23% ($P<0.05$) decrease in MDA levels. The activities of MnSOD and CAT were significantly ($P<0.001$) increased at 24.61% and 38.05% respectively while GPx decreased 46.96% in aged control rat brain when compared to young control rats. Decrease in MnSOD and CAT enzymes at 13.09% ($P<0.01$),

Table 1: Effect of T. chebula on brain mitochondria of young and aged rats

Parameters	Young		Aged	
	Control	Treated	Control	Treated
MAO-B	17.23 ± 1.08	16.87 ± 1.14	49.27 ± .83[a]***	22.78 ± 1.29[b]***
MDA	3.78 ± 0.28	3.28 ± 0.31[a]*	5.23 ± 0.27[a]***	4.27 ± 0.33[b]***
MnSOD	1.44 ± 0.10	1.35 ± 0.09	1.91 ± 0.12[a]***	1.66 ± 0.12[b]**
CAT	1.84 ± 0.14	1.65 ± 0.18	2.97 ± 0.18[a]***	2.06 ± 0.16[b]***
GPx	3.45 ± 0.29	3.62 ± 0.27	1.83 ± 0.22[a]***	3.88 ± 0.31[b]***

*Values are expressed as mean ± SD of six rats. Units - MAO-B, µmoles benzaldehyde semicarbazone formed/ hour/3mg protein; MDA, nmoles MDA formed/ mg protein; MnSOD, 50% reduction of NBT/min/mg protein; CAT, µmoles H_2O_2 consumed /min/mg protein; GPx, µmole GSH utilized/min/mg protein. [a]compared with Young control; [b]compared with Aged control; *P<0.05, **P<0.01, ***P<0.001.*

30.64% (*P*<0.001) respectively and increase the GPx activity at 52.84% (*P*<0.001) was observed after treatment with *T. chebula* aqueous extract to aged rats.

DISCUSSION

The neural degeneration occurring in aging rat brain induces a reduction in the level of neurotransmitters and the enzymes involved in their synthesis. In recent years, catecholamines manifest a crucial status among the sources of reactive oxygen species. The reaction of MAO yields H_2O_2, which also leads to the further generation of hydroxyl radical, the most powerful oxidant in cellular systems (26). In the present study an age-related increase in brain MAO-B activity was found in the aged control rats. Saura et al (1994) also demonstrated similar age dependent increases in brain MAO activities (27). Supplementation with *T. chebula* showed a significant reduction in MAO-B level in aged drug treated rats compared with aged control rats. The extract of *T. chebula* was reported to have scavenging activity toward hydroxyl radical (12). Also flavones and flavonols are reported to inhibit MAO-B. These compounds reduce the catabolism of dopamine and suppress the generation of endogenous neurotoxic radicals (28).

The present study also showed the increased level of lipid peroxidation byproduct MDA in brain of aged control rats compared with young control rats. Lipid peroxidation is one of the prominent organic expressions of oxidative stress induced by reactive oxygen species. Changes in catecholamine metabolism also caused pathological alterations in brain (29). *T. chebula* treatment restored the MDA level to young control levels in aged rats brain.

Imbalance between free radical production and catabolism of oxidant during aging would shift the cells towards oxidative stress resulting in alterations of membrane properties and cell dysfunction. MnSOD is one of the major mitochondrial antioxidant enzymes, which dismutates the superoxide radical to oxygen and hydrogen peroxide. In the present study, the increment in MnSOD activity with age could reflect a chronic adaptation to the increase in superoxide production. Santiago et al (1993) also showed an increase in SOD in the brain regions of aged rats (30). There is strong evidence that the oxidative metabolic pathway of catecholamines exert their neurotoxic effect mainly due to the generation of highly reactive quinines and superoxide radicals (31). The decrease in MnSOD activity in aged rats on *T. chebula* treatment may be due to the potential quenching of free radicals by its phenolic acids, which significantly decrease the superoxide radicals level (32).

CAT catalyses the reduction of hydrogen peroxide to water and oxygen in mitochondria (33). According to our results, a significant increase in CAT activity in aged rat brain mitochondria when compared to young control rats might be the result of a greater availability of hydrogen peroxide as substrate. Treatment with *T. chebula* decreased the CAT activity in aged rats compared to young rats by its hydrogen peroxides decomposition property (12).

Also GPx in brain may be the disposal of organic peroxides and the maintenance of protein thiols in their reduced states (34). In the present study, GPx activity was decreased in aged rat brain compared to young rats. This decrease may be due to the accumulation of the superoxide anion which inactivates GPx by reacting with the selenium at the active site of the enzyme (35). *T. chebula* administration increased the GP_X activity might be due to the protection of sulphydryl groups in glutathione from oxidative damages (12).

In conclusion, the administration of *T. chebula*

aqueous extract caused a decrease in MAO-B, MDA, MnSOD, CAT and changes in GPx through its compounds with potent MAO inhibitory activity. This may be a possible source for the treatment and prevention of depression and for protecting brain tissues against oxidative stress in aged rats and benefits in improving the quality of life in senescence.

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