IJPT
1735-2657/07/62-197-201
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 6:197-201, 2007

RIDR

RESEARCH ARTICLE

Antioxidant Effect of *Terminalia chebula* Aqueous Extract on Age-related Oxidative Stress in Heart

RAMALINGAM MAHESH and VAVA MOHAIDEEN HAZEENA BEGUM

For author affiliations, see end of text.

Received May 21, 2007; Revised August 22, 2007; Accepted November 20, 2007

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

Reactive oxygen species (ROS) are generated via normal metabolic processes or as the products of exogenous insults. They are capable of damaging essential biomolecules and accelerating cancer, cardiovascular diseases and neurodegenerative diseases. In the present study, the antioxidant role of *Terminalia chebula* aqueous extract was evaluated against age-related oxidative stress in heart tissues of young and aged rats. Young and aged rats were treated with *T. chebula* aqueous extract at a dose of 200mg/kg body weight in 1.5ml sterile water orally for 4 weeks. Control young and aged rats were received sterile water only. In aged rats, the increased content of malondialdehyde (MDA) was observed. The antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities, reduced glutathione (GSH), vitamin C and E levels were decreased in heart tissues of aged control rats. Administration of *T. chebula* to aged rats prevented the depletion of SOD, CAT, GPx activities and GSH, vitamin C and E contents. Also, the level of MDA content was decreased in heart tissues. The results of the present study show that *T. chebula* aqueous extract modulates the activities of antioxidants and lipid peroxidation through the management of oxidant/antioxidant imbalance in rat heart tissues.

Keywords: *Aging, Antioxidants, Heart, Lipid peroxidation, Terminalia chebula*

In the phenomenon of aging, theoretical and experimental studies indicate that the increased generation of reactive oxygen species has an important role. Oxidative modification of the major cellular macromolecular components, such as lipid, protein and DNA can result in deterioration of cardiac performance, energy production, and vascular endothelial function resulting in aging and death [1,2]. Many of the significant age-related changes are exhibited in post-mitotic tissues such as brain, heart and skeletal muscle. Those tissues with few or no cellular division will be theoretically more susceptible to accumulative damage caused by reactive oxygen species (ROS) [3]. It is interesting to note that myocardial injury shares a major etiological mechanism for aging in that both involve an increase in ROS generation and oxidative stress [4-6]. The aging heart undergoes significant functional and structural alterations leading to atrophy and a compensatory hypertrophy, followed by myocardial fibrosis [7]. In addition, there is an age-related decline in the capacity to withstand stress, such as ischemia/reperfusion [8]. In its most severe form, cardiac decay results in congestive heart failure, one of the leading causes of death in people over the age of 65.

Mammalian tissues possess an enzymatic defense system, which protects against the ROS. Antioxidant defense prevents the formation of active oxygen radicals and lipoperoxides. The antioxidant capacity decreases with advancement of age [9]. The antioxidant defense system consists of free radical scavenging enzymes like superoxide dismutase, catalase, glutathione peroxidase and antioxidants such as reduced glutathione, vitamin C and vitamin E, etc. An imbalance caused by increased generation of free radicals and decreased functional efficiency of antioxidant defense system has been suggested to be one of the primary factors that contribute to the aging process [10].

Herbal medicines are being used more frequently for age-related dysfunctions. Evidences support the fact that the protective effects of high fruits and vegetables consumption reduce the risks of age-related diseases and cardiovascular diseases [11]. The plant-derived natural antioxidants can be combined in a prophylactic food against age-related diseases involving free radicals. If water- and lipid-soluble antioxidants are mixed, electron transfer between them may occur and levels of antioxidants are increased, which scavenge reactive oxygen species [12,13].

Table 1. Effect of *T. chebula* treatment on heart enzymatic antioxidants of young and aged rats.

	Group I	Group II	Group III	Group IV
SOD	8.06 ± 0.74	9.22 ± 0.56 [a*]	5.44 ± 0.44 [a***]	7.78 ± 0.53 [b***]
CAT	46.34 ± 3.41	48.29 ± 3.26	39.36 ± 3.39 [a**]	46.22 ± 3.28 [b**]
GPx	5.23 ± 0.38	5.56 ± 0.24	3.63 ± 0.26 [a***]	5.16 ± 0.29 [b***]

Values are expressed as mean ± SD of six rats. Treatment of groups: Group I: Young control; group II: Young treated; group III: Aged control; group IV: Aged treated. Comparisons are made: [a] with group I; [b] with group III. Units: SOD: 50% nitroblue tetrazolium reduction/min/mg protein; CAT: µmoles of H_2O_2 consumed/min/mg protein and GPx: µmoles of GSH oxidized/min/mg protein. Symbols represent statistical significance: * $p< 0.05$; ** $p< 0.01$; *** $p< 0.001$

Chebulic myrobalan (*Terminalia chebula* Retz.) belonged to the family Combretaceae, known as 'Kadukkai' in Tamil is a native plant in India and found in the deciduous forests. Its dried ripe fruit tissues have traditionally been used to treat various ailments in Asia. It is a carminative, deobstruent, astringent and expectorant agent [14]. Its principle constituents contain chebulagic, chebulinic acid and corilagin [15]. A ellagitanninterchebulin along with punicalagin, terflavin-A, shikimic, gallic, tricontanoic and palmitic acids, beta-sitosterol, daucosterol, triethyl ester of chebulic acid and ethyl ester of gallic acid, a triterpene -chebupentol and arjungenin, terminoic acid, arjunolic acid, phloroglucinol, pyrogallol, ferulic, vanillic, p-coumaric and caffeic acids were isolated in fruits [16]. The carbohydrates such as glucose, sorbitol, fructose, sucrose, gentiobiosis, arabinose, maltose, rhamnose and xylose were also present in the fruits of *T. chebula* [14].

Chebulic myrobalan is highly nutritious and could be an important source of dietary supplement in vitamin C, energy, protein, amino acids and mineral nutrients [17]. *T. chebula* has been reported to exhibit a variety of biological activities, including anti-bacterial [18], anti-candidal [19], anti-anaphylaxis [20], anti-caries [21], and cardioprotective [22,23] activities. It also showed an antioxidant activity against gamma-radiation [24,25], cancer [26], hyperglycemia, diabetes mellitus [27,28], mutagenesis [29], hypercholesteolaemia and arteriosclerosis [30].

The present study was aimed to evaluate the antioxidant role of *Terminalia chebula* aqueous extract evaluated against age-related oxidative stress in heart tissues of young and aged rats.

MATERIALS AND METHODS

Preparation of T. chebula aqueous extract

The fruits of *T. chebula* were ripen from November to March and fall. The fully ripe fruits were collected from Kolli hills, Tamilnadu, India during the month of January 2005 from the ground as soon as they had fallen and shade dried. Hundred grams of dried fruit skins were hammered in to small pieces and followed by extraction with 800 ml distilled water for 24 h in water bath at 40°C, which was repeated for two times. The final yield of the aqueous extract was measured and used for treatment of experimental rats.

Dosage fixation

Various doses of *T. chebula* aqueous extract (50 mg, 100 mg, 200 mg, 300 mg and 400 mg/kg body weight) were used once daily for 4 weeks in 22-24 months aged Wistar rats (380-410 g) to assess the effective dose of the extract and duration of treatment against aging based on the contents of brain lipid peroxidation (LPO) and reduced glutathione (GSH). Pretreatment with *T. chebula* aqueous extract at doses of 200 mg, 300 mg and 400 mg/kg body weight for 4 weeks were found to be effective in aged rats. The minimal effective dose 200mg/kg dose was fixed as therapeutic dosage for the subsequent studies.

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

Tissue preparation

On completion of the 4 weeks, animals were anaesthetized with thiopenton sodium (50 mg/kg), heart was excised immediately, and immersed in physiological saline. For the preparation of heart homogenates (1 g of tissue plus 10 ml homogenization buffer), the frozen pieces were thawed on ice and then homogenized.

Assay of oxidation products

Lipid peroxidation was assessed by determining the level of malondialdehyde (MDA) in heart homogenates by the spectrophotometric method of Beuge and Aust (1978); the results were expressed as nmoles of MDA formed/mg protein using 1,1,3,3-tetraethoxypropane as

Table 2. Effect of *T. chebula* treatment on heart non-enzymatic antioxidants of young and aged rats.

	Group I	Group II	Group III	Group IV
GSH	7.08 ± 0.36	7.74 ± 0.39 ^{a*}	4.17 ± 0.32 ^{a***}	6.99 ± 0.38 ^{b***}
Vitamin C	2.47 ± 0.11	2.62 ± 0.09	1.26 ± 0.13 ^{a***}	2.44 ± 0.10 ^{b***}
Vitamin E	1.55 ± 0.09	1.61 ± 0.06	0.98 ± 0.11 ^{a***}	1.33 ± 0.13 ^{b***}

Values are expressed as mean ± SD of six rats. Treatment of groups: Group I: Young control; group II: Young treated; group III: Aged control; group IV: Aged treated. Comparisons are made: ^{a} with group I; ^{b} with group III. Units: GSH: μg/mg protein; Vitamin C: μg/mg protein and Vitamin E μm/mg protein. Symbols represent statistical significance: * $p< 0.05$; ** $p< 0.01$; *** $p< 0.001$

standard [31]. The protein carbonyl (PCO) content was analyzed using 2,4-dinitrophenylhydrazine (DNPH) as described by Levine *et al*. (1990) [32].

Assay of enzymatic antioxidants

Cardiac superoxide dismutase (SOD) activity was measured by the method of Kakker *et al*. (1984) using NADH-PMS-NBT [33]. Catalase (CAT) activity was measured by the method of Beers and Sizer (1952) 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^{-1} [34]. Glutathione peroxidase (GPx) was estimated by the method of Rotruck *et al*. (1973) [35]. Protein concentrations were assayed by the method of Lowry using bovine serum albumin as a standard [36].

Estimation of non-enzymatic antioxidants

Reduced glutathione (GSH) was measured as described by Ellman (1959) using 5, 5-dithiobis- (2-nitrobenzoic acid) (DTNB) reagent [37]. Ascorbic acid (vitamin C) and α-tocopherol (vitamin E) contents were assayed according to Omaye *et al*. (1979) and Desai (1984) respectively [38,39].

Statistical analysis

The values are expressed as mean ± standard deviation (SD). The results were computed statistically (Graphpad Instat) using one-way analysis of variance. Post hoc testing was performed for inter-group comparisons using the least significance test.

RESULTS

The level of heart MDA was significantly increased 41.42% in aged rats compared with young rats as shown in Fig 1. In *T. chebula*-treated rats, level of heart MDA was significantly decreased (p<0.001) in comparison with age-matched controls. In drug-treated young rats, a 14.92% (p<0.05) decrease in MDA levels was observed. The respective values for MDA content were 1.81±0.14 nmoles of MDA formed/mg protein for young control rats, 1.54±0.12 nmoles of MDA formed/mg protein for young extract-treated rats, 3.09±0.17 nmoles of MDA formed/mg protein for aged control rats and 2.03±0.14 nmoles of MDA formed/mg protein for the aged extract-treated rats.

The activities of the heart antioxidant enzymes SOD, CAT and GPx were significantly decreased in aged control rats when compared to young control rats (Table 1) at 32.51%, 15.06%, and 30.59% respectively. *T. chebula* aqueous extract treatment in aged rats showed increases in the antioxidant enzymes at 30.08%, 14.84%, and 29.65% respectively. *T. chebula*-treated young rats showed 12.58% increase in SOD activity.

Table 2 shows a significant decrease in GSH, vitamin C and E level observed in heart of aged control rats as 41.10%, 48.99% and 36.77% respectively. *T. chebula* treatment increase the GSH content in aged rats at 40.34 % and increase the GSH content in young rats at 8.53%. Also the levels of vitamin C and E were significantly increased (p<0.001) in comparison with age-matched controls.

DISCUSSION

For many years, it has been proposed that the life long production and accumulation of free radicals as by-product of oxidative metabolism is the basis of aging process [40]. In the present study, the increase in MDA content was observed in the hearts of aged control rats compared to young control rats. Muscari et al. (1990) proposed that the increase in MDA levels is the final products of lipid peroxidation which are metabolized in the cells [41]. Others showed several damages in the aged heart caused by free radical generation [7], such as greater formation of both hydrogen peroxide and 8-hydroxy-2-deoxyguanosine. Our results are in agreement with that of other authors [42,43], who found an increase in MDA levels in the heart, which affects the membranes and is responsible for the age-related changes. In animals treated with aqueous extract of *T. chebula*, there was considerable decrease in the level of MDA, suggesting a decrease in lipid peroxidation and protects the myocardium from oxidative damage. Flavonoids can make complexes with metals, and inhibit metal-initiating lipid peroxidation [44].

The endogenous free radical scavenging enzyme, SOD specifically dismutates superoxide radicals in tissues. Our results indicated that heart SOD activity was decreased in aged rats compared to young control rats. Such a reduction could be attributed to an increase in hydroxyl radical and hydrogen peroxide levels [45]. Supplementation of *T. chebula* to aged rats increased the heart SOD activity to the levels of young control rats. This may be attributed by scavenging superoxide

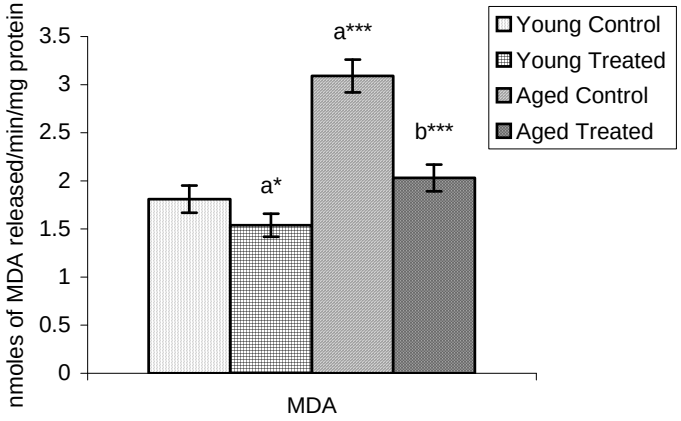

Fig 1. Effect of *T. chebula* treatment on heart malondialdehyde (MDA) in young and aged rats. Values are expressed as mean ± SD of six rats. Comparisons are made: [a] with with young control group; [b] with with aged control group. * $p<$ 0.05; ** $p<$ 0.01; *** $p<$ 0.001.

radicals, hydrogen peroxides and inhibiting oxidative stress in aging [44].

Catalase plays a minor role if any, in detoxificaiton of H_2O_2 in the heart relative to glutathione peroxidase, as compared with other tissues [46]. In the present study, the activities of catalase and glutathione peroxidase in aging heart were significantly low compared to young rats. This may indicate that these antioxidant enzymes are responsible for the increased hydrogen peroxide concentration in heart tissues, thus, emphasizing its role in the control of cellular lipid peroxide concentration [47]. An activation of catalase and glutathione peroxidase was observed due to the protective action of the extract from *T. chebula* fruits. Administration of *T. chebula* extract brought down the oxidative stress substantially and decreased the MDA levels in the aging myocardium. This property may be attributed by the phytochemicals present in *T. chebula*. These antioxidant activities of the plant derived compounds are based on hydrogen donation abilities and chelating metal ions [48].

Not only is the level of the protective enzymes, but also that of non-enzymatic antioxidants (GSH and vitamins) involved in preventing peroxidative attack in various tissues. Aged rats (rats between 4 and 24 months of age) showed significant reduction in concentration of cellular GSH. This result is in harmony with those obtained from Stochs *et al.* [49]. The lower content of heart tissue GSH in aged rats could be due to their participation in many detoxification reactions to protect against free radical injury and oxidative tissue damages of aged rats. *T. chebula* administration to aged rats showed marked increase in GSH levels.

In addition to GSH, vitamins C and E are interrelated by recycling processes [50]. Recycling of tocopheroxyl radicals to tocopherol is achieved by reaction with ascorbic acid [51]. Dehydroascorbic acid is formed in reaction with reduced GSH [52]. McCay *et al.* (1989) have shown the presence of a liable glutathione dependent factor, which cycles the tocopheroxyl radicals to tochopherol [53]. If recycling of tocopheroxyl radicals to tocopherol is a major mechanism for maintenance of tissue tocopherol levels, deficiency of ascorbic acid is expected to result in depletion of tissue tocopherol. In the present study, vitamins C and E levels were decreased in heart of aged rats compared with young control rats. Recycling of tocopheroxyl radicals to tocopherol may have been hindered, resulting in elevated lipid peroxidation reactions. Supplementation with *T. chebula* increased the heart vitamin E and C in aged rats. This shows the efficacy of *T. chebula* in enhancing heart functions by its phytochemicals such as flavonoids and ascorbic acid derivatives.

The present study concludes that the repeated treatment of aged rats with *T. chebula* aqueous extract might result in the decrement of free radical production that is described by a marked reduction in heart MDA level and increments in heart enzymatic as well as non-enzymatic antioxidants in heart tissue. This modulatory activity of *T. chebula* treatment may be due to its natural antioxidant phytochemicals such as flavonoids, tannins, polyphenolic acids and ascorbic acid derivatives.

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CURRENT AUTHOR ADDRESSES

Ramalingam Mahesh, Department of Siddha Medicine, Faculty of Science, Tamil University, Vakaiyur, Thanjavur – 613 010, Tamilnadu, India. Email: melanimahesh@gmail.com (Present Address: Department of Pharmacology, School of Dentistry, Kyung Hee University, Seoul 130-701, Republic of Korea).

Vava Mohaideen Hazeena Begum, Department of Siddha Medicine, Faculty of Science, Tamil University, Vakaiyur, Thanjavur – 613 010, Tamilnadu, India. Email: drvhazeenabegum@gmail.com (Corresponding author).