OPTIMIZING ACTIVITY OF *ASPARAGUS RACEMOSUS* ON PLASMA LIPID PEROXIDATION AND ANTIOXIDANT IN AGED RATS.

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Summary

The "free radical theory of aging" proposes that aging occurs as a consequence of the deleterious effects of free radicals produced during normal cellular metabolism. The free radical-mediated lipid peroxidation has been proposed to be critically involved in several diseases. Antioxidant therapy has gained an utmost important in the treatment of free radical mediated diseases. Current research is now directed towards finding naturally occurring antioxidant of plant origin. In Indian system of medicine *Asparagus racemosus* is an important medicinal plant and its root paste or root juice has been used in various ailments and as health tonic. In the present study, we have determined the plasma levels of lipid peroxidation expressed as Malondialdhyde (MDA) and activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non enzymatic antioxidant including vitamin E, C and reduced glutathione content were determined. In aged rats, enzymatic and non-enzymatic antioxidants were low whereas the lipid peroxidation rate, as revealed by MDA content, was found to be high. Supplementation of *Asparagus racemosus* root extract (ARRE) to aged rats restored the activities of enzymatic and non-enzymatic antioxidant to near normal and decreased the MDA content. These results confirm that ARRE strengthened the endogenous antioxidant defense and thereby alleviate the age associated increased free radicals.

Key words: *Asparagus racemosus;* Antioxidant; Lipid peroxidation; Aging

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Introduction

Aging is characterized by slow, progressive, structural and functional changes that take place at cellular, tissue and organ level. These changes resulting in gradual functional decline, decreased adaptability and ability to face stress and increased probability of age associated diseases [1]. The free radical theory of aging proposes that aging occurs as a consequence of the deleterious effects of free radicals produced during normal cellular metabolism [2,3]. Polyunsaturated fatty acids (PUFAs) are easily susceptible to peroxidation. Plasma lipids are increased progressively as a function of age in animals [4]. It has been reported that the peroxidizability index of plasma lipids was increased in aged rats [5].

Free radical react with lipids and cause peroxidative changes that result in enhanced lipid peroxidation. Lipid peroxidation is initiated by the abstraction of a hydrogen atom from polyunsaturated fatty acid side chain. The free radical-mediated lipid peroxidation has been proposed to be critically involved in several diseases including cancer, cardiovascular diseases, brain dysfunction, and degenerative process associated with aging [6]. In treatment of theses diseases, antioxidant therapy has gained an utmost importance. Antioxidants reported to prevent oxidative damage by free radical and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers [7,8].

Living organisms have developed complex antioxidant systems to counteract reactive oxygen species. These antioxidant systems include enzymes such as superoxide dismutase, catalase and glutathione peroxidase; macromolecules such as albumin, ceruloplasmin and ferritin; and a variety of small molecules, including ascorbic acid, alpha-tocopherol, reduced glutathione, methionine, uric acid and bilirubin [9]. Every living organism has antioxidant defense to cope up with the ROS. However, since this enzyme defense system is not hundred percent efficient in aging due to high levels of free radicals, the entire array of available endogenous antioxidant enzymes cannot fully neutralize the ROS. Supplementations of exogenous antioxidants which full fill this gap and completely neutralize the ROS. Current research is now directed towards finding naturally occurring antioxidant of plant origin. Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability [10]. Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. [11].

In Indian system of medicine *Asparagus racemosus* Willd root (Liliaceae) (Eng: Willd asparagus, Tamil: Thanner Vittan Kizhangu,) is an important medicinal plant. Traditionally it is used as health tonic and common Indian home remedy used as a rejuvenator, promoter of strength, breast milk and semen [12]. Roots of the plant have been used in the Indian traditional system of medicine for the treatment of various ailments in human being [13]. *Asparagus racemosus* is a well known ayurvedic rasayana which prevent ageing, increase longevity, impart immunity, improve mental function and add vigor and add vitality to the body and also used in nervous disorders, dyspepsia, tumors, inflammation, hyperdipsia, neuropathy, hepatopathy [14]. *Asparagus rasemosus* has also been reported to have potent adaptogenic activity [15] and antioxidant property [16]. Previously reported that ARRE contain saponin [17], alkaloids [18], polysaccharide [16], polyphenols, flavonoid and vitamin-C [19,20]. Choudhary [21] showed that the mineral content of *Asparagus racemosus* root contain calcium, manganese, magnesium, potassium, copper, zinc and cobalt Therefore, our study was concentrated on the role of *Asparagus racemosus* in augmenting the functions of plasma antioxidants and determines the level of lipid peroxidation in aged rats.

Methods

Animals

Male albino rats of wistar strain approximately 3-4 months old rats weighing approximately 140-160g (young) and 24-26 months old rats weighing approximately 380-410g (aged) were used in this study. They were healthy animals obtained from Sri Venkateswara enterprises, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºc and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water *ad libitum*. They were acclimatized to the environment for 1 week prior to experimental use. The study protocol was carried out as per the rules and regulation of the institutional animal's ethics committee (IAEC).

Plant Material

The roots of the *Asparagus racemosus* were collected from the kolli hills, Tamil Nadu, South India. The collected roots were identified and authenticated by a botanist Prof. Dr. M. Jegadesan, Department of Herbal and Environmental science, Tamil University, Thanjavur, Tamil Nadu. A Voucher specimen (Specimen no: 29) has been deposited at the Herbarium of the department. The roots were cut into small pieces and shade dried and powdered finely then used for extraction.

Preparation of plant extract

A required quantity of the powder (5g) was suspended in a measured amount of distilled water (600ml). The suspension was boiled until the quantity was reduced to 100ml. The resultant decoction was cooled and used in the present study. The concentration of resultant decoction was 50 mg/ml. For experiments 500mg/kg body weight of *Asparagus racemosus* root extract (ARRE) was used. This effective dose of ARRE was selected based on the dose dependent studies carried out in aged rats [22].

Experimental Design

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows.

Group I	:	Control young rats
Group II	:	Young rats administered *Asparagus racemosus* root extract (ARRE) (500mg/Kg b.wt/day) orally for four weeks
Group III	:	Control aged rats
Group IV	:	Aged rats administered *Asparagus racemosus* root extract (ARRE) (500mg/Kg b.wt/day) orally for four weeks.

After the completion of experimental regimen, the rats were fasted over night and blood samples were collected by cervical decapitation. Plasma was separated and used for various biochemical estimations.

Biochemical Estimations

MDA released from endogenous lipoperoxides, reflecting the lipid peroxidation process, were assayed in plasma as described by Beuge and Aust [23]. The activities of antioxidant enzymes Cu/Zn SOD, Catalase and Glutathione peroxidase were determine by the method of Kakkar et al. [24], Beers and Sizer [25] and Rotruck et al. [26] respectively. The levels of non-enzymatic antioxidants such as GSH, Vitamin C and Vitamin E were estimated by the method of Moron et al. [27], Omaye et al. [28] and Baker et al. [29] respectively.

Statistical analysis

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons [30]. Statistical analysis carried out by Ms-Windows based graphpad Instat software (GraphPad Software, San Diego, CA, USA) 3 version was used and $p< 0.05$; and $p< 0.001$ were considered to be significant.

Results

The Group 3 aged rats showed a significant increase in plasma MDA and decreases in the activity of SOD, CAT and GPx as compared to Group 1 young control rats (Table 1). The increase was 34.38% for MDA and decrease 34.72 %for SOD, 36.76 %for CAT and 24.36%for GPx in aged rats. ARRE treated Group 4 aged rats showed a significant reduction in MDA level and increase in the activities of SOD, CAT and GPx as compared to Group 3 aged control rats. The decrease was 27.16% for MDA and increase 31.88 %for SOD, 31.75 %for CAT and 21.33 %for GPx in aged rats treated with ARRE.

Table 1. Effect of *Asparagus racemosus* on plasma SOD, catalase and Glutathione peroxidase in control and experimental animals

Parameters	MDA (nmole/L)	(SOD) (U/ml)	Catalase (U / ml)	Glutathione peroxidase (U / ml)
Young control	2.73 ± 0.11	1.44 ± 0.07	0.68 ± 0.02	0.78 ± 0.04
Young +ARRE	2.66 ± 0.17	1.48 ± 0.06	0.72 ± 0.04	0.81 ± 0.03
Aged control	4.16 ± 0.14[a*]	0.94 ± 0.07[a*]	0.43 ± 0.03[a*]	0.59 ± 0.03[a*]
Aged + ARRE	3.03 ± 0.15[b*]	1.38 ± 0.08[b*]	0.63 ± 0.04[b*]	0.75 ± 0.05[b*]

Values are expressed as mean ± S.D. for six rats in each group.
[a]As compared with Young control rats; [b]As compared with Aged control rats. [*]$p<0.001$.

The Group 3 aged control rats showed a significant decrease in plasma GSH, Vitamin C and Vitamin E as compared to Group 1 control young rats (Table 2). The decreases being 30.37 % for GSH, 33.83 %for Vitamin C and 31.97 %for Vitamin E in aged rats. ARRE treated Group 4 aged rats showed a significant increases in GSH, Vitamin C and Vitamin E as compared to Group 3 aged control rats. The increases being 30.09 % for GSH, 30.71 %for Vitamin C and 30.25 %for Vitamin E in aged rats treated with ARRE. In young rats Group 2 ARRE administration showed a lowered MDA and increased GSH content and non-significant changes (NS) in all other parameters as compared to Group 1 young control rats.

Table 2. Effect of *Asparagus racemosus* on plasma Reduced glutathione (GSH), Vitamin C and Vitamin E in control and experimental animals

Parameters	GSH (mg/dl)	Vitamin C (mg / dl)	Vitamin E (mg/dl)
Young control	12.38 ± 0.67	1.33 ± 0.06	1.22 ± 0.04
Young + ARRE	13.63 ± 0.69[c**]	1.39 ± 0.04	1.27 ± 0.03
Aged control	8.62 ± 0.67 [a *]	0.88 ± 0.04 [a *]	0.83 ± 0.03[a *]
Aged + ARRE	12.33 ± 0.68 [b *]	1.27 ± 0.06 [b *]	1.19 ± 0.03 [b *]

Values are given as mean ± S.D. for six rats in each group.
[a]As compared with Young control rats; [b]As compared with Aged control rats.
[c]As compared with Young control rats.
** $p< 0.05$, * $p<0.001$.

Discussion

Oxidative stress is considered to have a critical role in changes associated with senescence and it is conceivable that antioxidants are important anti-aging agents [31]. The intensity of oxidative stress is determined not only by the free radicals production but also by antioxidant (enzymatic and non enzymatic) defense [32]. Plasma plays a central role in the transport and fate of lipids, and thus potentially susceptibly to lipid peroxidation and the relative contributions of each of the various endogenous antioxidants in preventing peroxidative damage to lipids. Furthermore, the fate of lipid hydroperoxides once they have been formed in plasma or taken up into it and the possible involvement of the various antioxidants. The formation and degradation of lipid hydroperoxides in plasma is relation to the consumption of endogenous antioxidants [33].

Malondialdehyde (MDA), a commonly used biomarker of lipid peroxidation, belongs to the group of aldehydes arising mainly from lipid peroxidattion in the body. Measured levels of MDA can be considered an indirect index of oxidative injuries associated with lipid peroxidation [34]. An age associated increase in lipid peroxidation was observed in our present study. Lipid acts as vital substrates for lipid peroxidattion and the enhancement of lipid profile during aging [4] may be the cause for increased lipid peroxidation. Also, an enhanced level of lipid peroxides in hyperlipidaemia was reported [35], suggesting a causal relationship between lipids and lipid peroxidation. Some investigators have reported that plasma lipid peroxides increased in experimental animal with age [5] may be due to increased plasma lipids. Supplementation of ARRE to aged rats decreased the levels of

lipid peroxide. Our findings are in concordance with Visavadiya and Narasimhacharya [20] study, which reported that supplementation of *Asparagus racemosus* root powder decrease plasma lipid peroxidation. ARRE treated with young rats significantly decrease in lipid peroxidattion level than of young control rats.

A broad class of protective agents termed antioxidants, which prevents oxidative damage by reacting with free radicals before any other molecules can become a target neutralizes the harmful effect of reactive oxygen species. The enzymatic antioxidant and non-enzymatic antioxidants, which play an important role in the protection of cells against free radical mediated damage [36]. During aging, antioxidant functions decline in almost all mammals [37]. Also, higher levels of free radicals have been reported in aged rats [38].

In the present study, decline in the activities of plasma SOD and CAT in aged rats as compared to young control rats. The possible reason could be the inactivation of this enzyme due to high level of free radical during aging. The age-related decrease in the activity of SOD and CAT in our study is in corroborated by earlier investigations [39]. Andersen *et al*. [40] reported that there was age related decrease in SOD activity. Inactivation of SOD has been reported to be associated with aging in rats due to its product, hydrogen peroxide [41]. Another explanation for the decrease in SOD activity is as a function of age could be an increase in glycation of SOD [42]. The decline in the activity of catalase due to free radicals generated during aging [38]. The superoxide radical could also inhibit the activity of catalase [43]. Decline of SOD and CAT activity in aged rat was brought back to a normal level with administration of ARRE. This is may be due to the free radical scavenging activity of phyrochemicals such as flavonoid and vitamin C in the ARRE [19] and thereby preserve the enzyme activity. ARRE treatment showed insignificant changes in SOD and CAT activity when compare to young control rats.

The decreased activity of plasma GPx was observed in aged rats may be attributed to the decline in glutathione level. Our finding is also in agreed with Itoy *et al* [44] study. Increased availability of substrate (glutathione) influenced the activity of GPx in plasma during ARRE treatment. ARRE treatment with young rats no changes in GPx activity when compared to young control rats.

Glutathione is an important antioxidant that functions directly in elimination of toxic peroxides and aldehydes and indirectly in maintaining vitamins C and E in their reduced and functional forms. Vitamin C deficiency results in decreased plasma GSH and vitamin E supplementation increases plasma GSH. Both vitamin C and E concentrations in plasma decrease with age [45], suggesting that GSH may also decrease in plasma with age Diminished GSH status has been linked with normal aging as well as with neurodegenerative disease [46]. The finding of our study showed decreased level of plasma glutathione in aged rats as compared to young rats. This may be due to increased utilization for detoxification of free radicals generated during aging. Administration of ARRE showed significant enhancement in the level of glutathione in aged treated rats (group IV) when compared with aged control rats (group III). Antioxidant property of ARRE might be attributed to the phenolic compounds present. Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants [47]. GSH deficiency is often

accompanied by lower levels of α-tocopherol and ascorbate, and diminished activity of glutathione peroxidase, catalase, and superoxide dismutase, all of which are reversible when GSH level is normalized [48]. In the present study, ARRE increase the GSH content in aged rats.

Ascorbate plays an important role with the lipophilic antioxidant α-tocopherol in protecting the membrane from oxidative stress. Recycling of ascorbic acid requires GSH, which reduces dehydroascorbate to ascorbate [49]. Ascorbate in turn is essential for the recycling of tocopherol radical to tocopherol [50]. The observed decline in glutathione level may contribute to the decrease in ascorbate as well tocopherol concentration in aged rats. Both vitamin C and E concentrations in plasma decrease with age reported by Samiec *et al*. [45] studies. In the present study, we also observed the decreased level of plasma vitamin C and Vitamin E in aged rats, demonstrating the increased free radicals accumulation in aging. Supplementations of ARRE to aged rats improved the Vitamin C and E level as compared to aged control rats, which may be due to the presence of vitamin C and polyphenolic component in ARRE. Earlier reports suggest that polyphenols may regenerate a-tocopherol through reduction of the a-tocopheroxyl radical [51]. ARRE treatment to young rats maintained the normal level of vitamin C and E.

The results of the present study indicate that the optimizing activity of ARRE in aged rats may probably related to a counteraction of free radicals by its antioxidant nature of ARRE, to strengthening endogenous antioxidant defense by its ability to increase the non enzymatic antioxidants like GSH, vitamin C, vitamin E and decreased content of lipid peroxide which is used as a marker for oxidative stress. This optimizing activity of ARRE mainly attributed to the presence of enriched therapeutic phytochemical constituents, which act synergistically to alleviate the age associated increased free radicals and thereby decrease oxidative stress.

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