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Research Article

Effect of Shallot (Allium cepa var. Aggregatum) Supplementation on Antioxidant Status of Cigarette Smokers

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Abstract

Shallot is a rich source of natural antioxidant components. The objective of the study was to evaluate the antioxidant potential of shallot in habitual cigarette smokers. Subjects were 40 healthy males (25-40 years) with smoking history of >5 cigarette per day for at least 3 years. They were divided into 2 groups of 20 each, experimental and control. Control and experimental group received placebo and 500 mg shallot capsules twice a day respectively for one month. Antioxidant activity, lipid peroxidation and total thiol was measured in serum of subjects before and after supplementation. Results showed a highly significant increase in total thiol and total antioxidant activity in experimental group in comparison to placebo. There was also a significant decrease in serum lipid peroxide, which was 4.9 and 4.79 at the beginning of the study and decreased to 4.3 and 4.71 in experimental and control group respectively at the end of the study. Negative correlation was observed between number of cigarettes per day and serum total thiol indicating that when the number of cigarette smoked per day increases, serum total thiol decreases (R^2=0.130). A negative correlation was observed between total thiol and lipid peroxidation which showed that when oxidative stress was more, the total thiol molecule reduced due to the oxidation (R^2=0.369). Correlation between serum lipid peroxidation and number of cigarette per day was found to be positive, (R^2=0.235). In conclusion, shallot supplementation improved the antioxidant status in cigarette smokers.

Keywords: Total Thiol; Serum Lipid Peroxides; Total Antioxidant Capacity; Positive Correlation

Introduction

Cigarette smoke, as a pollutant has been established to include a variety of xenobiotics, some of which are known to be oxidant or free radicals that can directly and indirectly initiate and propagate the process of lipid peroxidation [1]. The enhanced susceptibility of erythrocytes of smoker's to peroxidation may reflect the lower activities of glucose-6-phosphate dehydrogenase and glutathione peroxidase. Decreased activity of glucose-6-phopspahete dehydrogenase can be caused by extracellular or intracellular lipid hydroperoxides [2]. Differences in glutathione peroxidase activity between smokers and non-smokers have been reported previously and maybe associated with decreased selenium status [3]. Thus, it is believed that smokers encounter a sustained free radical load. It has been shown that cigarette smoking caused an increase in blood and serum malonaldehyde content. Many studies have shown an increased lipid peroxidation due to cigarette smoking [4,5].

Allium families are reported with high antioxidant potential. Shallot belonging to the family Alliaceae is one of the promising plants which demonstrates significant antioxidant as well as anti-inflammatory properties, useful in the protection of various diseases such as respiratory and nervous diseases. The important active compounds in Alliaceae family such as onion, garlic or shallot are different but they mainly contain total phenolic compound that have the -OH group [6]. In addition, most of the phenolic compounds such as furostane, saponins and high level of quercetin, isorhametin and other glycosides are present in shallot [7]. Our earlier study indicated that water extracts of shallot exhibited high

antioxidant activity ranging between 648,525 - 772,059 µmol/g. Dry shallot powder also had a very high soluble dietary fiber content [8]. As cigarette smokers are at high risk of oxidative risk, the present study was planned to evaluate the antioxidant potential of shallot in habitual cigarette smokers.

Methodology

The study involved recruiting subjects using inclusion and exclusion criteria as detailed below, administering dry shallot powder capsule or a placebo for a month and analyzing blood for lipid peroxidation before and after supplementation. The study approval was taken from Human Ethical Committee, University of Mysore.

Subjects

The antioxidant properties of shallot were studied in 40 human subjects. Subjects were volunteer Iranian male cigarette smokers with a history of smoking from past three years with minimum of 5 cigarettes per day. The age group of 25-40 years was included for the study. Subjects with any kind of disease or those who were on medication were excluded from the study. All participants were provided with specific written consents obtained prior to entrance into the study.

Study

Subjects were divided randomly into 2 groups (control and experimental). Five hundred mg of dry shallot powder (*Allium cepa* var. aggregatum) and placebo in the form of capsule were given to study group and control group respectively twice a day. Each individual was extensively interviewed for specific information like smoking, medications, history of specific disease before obtaining blood. The supplementation was given for a duration of one month. Before and after the study, blood samples were collected. Blood was analyzed for lipid peroxidation [9], total thiol [10], and total antioxidant capacity [11].

Analysis

- **Measurement of Lipid peroxidation level:** Lipid peroxidation level of plasma was determined by the reaction of thiobarbituric acid (TBA) with malonaldehyde and other lipid peroxides. To 0.5 ml serum, 2.5 ml of 20 mg/dl trichloroacetic acid is added and tubes are left to stand for 10 min at room temperature. After centrifugation at 3500 rev./min for 10 min, the supernatant is decanted and the precipitate is washed once with 0.05 M sulfuric acid. Then 2.5 ml of 0.05 M sulfuric acid and 3.0 ml of 0.2 mg/dl TBA in 2 M sodium sulfate are added to this precipitate and the coupling of lipid peroxide with TBA is carried out by heating in a boiling water bath for 30 min. After cooling in cold water, the resulting chromogen is extracted with 4.0 ml of n-butyl alcohol by vigorous shaking. Separation of the organic phase is facilitated by centrifugation at 3000 rev./min, for 10 min and its absorbance is determined at the wavelength of 530 nm. Malondialdehyde was used to produce standard calibration curve. And the determined values are expressed in the terms of malondialdehyde (nmol/ml) used as reference standard [9].
- **Measurement of Plasma Total Antioxidant Capacity (FRAP Assay):** Working FRAP reagent was prepared freshly by adding acetate buffer pH 3.6, 10mM TPTZ in 40 mM HCL and 20mM $FeCl_3.6H_2O$ in the ratio of 10:1:1. To 3.0 ml of working FRAP reagent, 100 ml of serum or standard was added in a test tube, vortex mixed and absorbance was read at 593 nm against a reagent blank at a predetermined time after mixing. Test was performed at room temperature and absorbance was read at 0-6 min. The results were calculated using the FRAP value of standard [10].
- **Measurement of Plasma Total thiol molecule:** Total sulfhydryl content was determined in plasma by the method of Hu and Dillard [11]. Am aliquot of plasma (0.2 ml) was mixed in a 10 ml test tube with 0.6 ml of the Tris-EDTA buffer followed by the addition of 40 µl of 10mM DTNB and 3.16 ml of absolute methanol. The test tube was capped and the color developed for 15-20 min, followed by centrifugation at 3500 rev./min for 10 min at ambient temperature. The absorbance of the supernatent was measured at 412 nm (A) and subtracted from a DTNB blank (B) and a blank containing the sample without DTNB (C) [(A-B-C) x (4.0/0.2)/13.6 = (AB-C) x1.47 mM].

Results and Discussion

The results of the study are compiled in Table 1 and figures 1-6. Table 1 presents the biochemical parameters of blood of control and experimental group.

Subjects were examined for serum total thiol before supplementation and it was found to be 0.146 µmol/ml in experimental group and 0.157 µmol/ml in control group. After administration of shallot capsules to experimental group and placebo to control group for a month, total thiol was 0.243 and 0.158 µmol/ml respectively. The total thiol protein of smokers increased significantly after 30 days of shallot supplementation. The difference was statistically significant for experimental group and non-significant for control group indicating the impact of shallot consumption on total thiol levels of subjects. Negative correlation was observed between number of cigarette smoked per day and serum total thiol levels indicating that when number of cigarettes smoked per day increases, serum total thiol decreases (Figure 1). It can be due to the increase in oxidation of thiol molecule.

Biochemical indices	Control group		Experimental group	
	Before	After	Before	After
Total Thiol (µmol/ml)	0.157 ± 0.046	0.158 ± 0.070 ns	0.146 ± 0.041	0.243 ± 0.073 ***
Serum lipid peroxidation (nmol/ml)	4.79 ± 0.355	4.71 ± 0.313 ns	4.83 ± 0.343	4.30 *** ± 0.329
Serum total antioxidant capacity (µmol/ml)	670.3 ± 14.40	669.0 ± 15.44 ns	658.6 ± 13.04	683.2 ± 17.55 ***

Table 1: Effect of shallot supplementation on biochemical parameters of subjects.

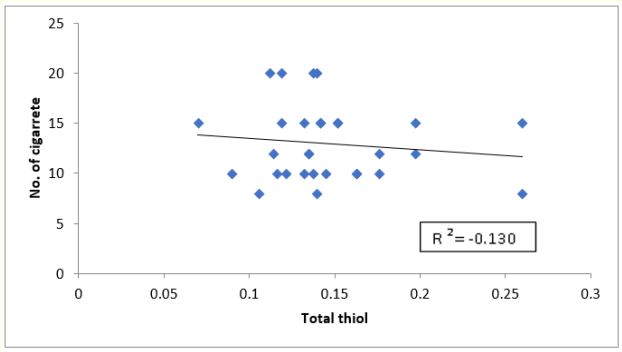

Figure 1: Correlation between total thiol and No. of cigarette per day.

The major cause of chronic oxidative stress in humans is exposure to free radicals in cigarette smoke. Cigarette smoke free radicals are considered an important cause of atherosclerosis and cancer [12,13]. Thiols are powerful reducing agents that are capable of acting as antioxidants *in vivo*. Thiols exist in three forms, free thiol and two types of disulfides, namely homodisulfides and heterodisulfides. Several aminothiols, example, cysteine, homocysteine (Hcy) and GSH, and disulfides (eg., cystine, homocystine, and oxidized glutathione), interact by means of redox, and disulfide exchange [14]. This dynamic system (with respect to thiol status) is important for normal physiologic function [15]. Changes in the redox thiol status lead to the induction of oxidative stress and apoptosis. As both an intracellular and extracellular redox buffer, t-SH plays important roles in prevention of atherosclerosis [16]. T-SH plays an important role in antioxidant reactions, and in catalysis, regulation and electron-transport reaction and in reaction that preserve the correct structure of proteins. Mixed disulfides with proteins are formed by reaction of S-thiolation, in which protein thiols conjugate with non-protein thiols [17]. This process plays a regulatory and an antioxidant role, since it protects protein-SH groups against irreversible oxidation from $-SO_2H$ and $-SO_3H$; moreover, it participates in signal transduction [18].

Maintaining the intracellular thiols, such as GSH, in their reduced form, may allow for the maintenance of plasma homocysteine and other intracellular thiols in redox states [19]. Since the plasma GSH concentration reflects its levels in various tissues, a reduced plasma concentration of GSH may be a diagnostic indicator of a pathological state [20]. GSH is the most important endogenous antioxidant in humans, it is often accompanying by other endogenous thiols, such as cysteine, cysteinyl glycine and even Hcy (in low concentration). These thiols scavenge ROS and are involved in preserving the pro-oxidant antioxidant balance in human tissue [21]. GSH is an abundant tripeptide that protects against oxidative stress and damage in nearly all cells and tissues [22,23]. It is a major intracellular antioxidant and functions by scavenging free radicals, detoxifying lipid peroxides via glutathione peroxidase, and conjugating reactive electrophilic toxicants and carcinogens. In addition, GSH in involved in numerous other cellular pathways including protein, and DNS synthesis, DNA repair, and immune surveillance. GSH is oxidized to its disulfide form (GSSG) but is subsequently reduced back to GSH by GSH reductase. An alternative pathway for GSSH metabolism is protein glutathiolation (also referred to as glutathionylation) where thiol-disulfide exchange occurs with cysteine (Cys) residues in proteins to from GSSP. The formation of GSSP within cells can be substantial and reach 200 µM in certain tissues [24]. There is considerable evidence that glutathiolation represents an important redox-sensitive regulator of cellular activities [17,25]. An induction of GSH synthesis may accompany a smoking related increase in GSH utilization though oxidation to GSSG and GSSP resulting in increase in both GSH an GSSP. This would suggest that GSSP-GSH ratios are less sensitive to oxidative stress than GSSP alone. The amount of protein that is glutathiolated in blood of smokers is reported to be high (ranging from 0.05 to 0.38 mmol/l) 34 to 43% higher than those observed in non-smokers. A dose response relationship is reported to be apparent between GSSP levels and tobacco smoke measurements such as cigarette smoked per day, blood cotinine (an alkaloid found in tobacco and also a metabolite of nicotine)) and

blood thiocyanate. A similar but smaller increase in plasma GSSP levels was also found in smokers compared with non-smokers [26]. These findings provide compelling evidence that blood GSSP is an indicator of oxidative stress and those abundant free radicals in cigarette smoke cause increases in blood GSSP concentrations. As shown in Figure 1. interestingly a negative correlation was observed between total thiol and number of cigarettes smoked.

Serum lipid peroxidation

As explained earlier, smoking also produces xenobiotics and toxic compounds in the body. Many researchers have also reported lower serum vitamin C content in smokers compared with non-smokers. Thus lipid peroxidation increases in smokers. Serum lipid peroxidation was found to be 4.9 and 4.79 at the beginning of study in experimental and control group respectively. After 30 days supplementation, estimation was repeated again and found to be 4.3 and 4.71 in study and control group respectively. Statistical analysis showed a significant reduction in the serum lipid peroxidation level of subject after shallot supplementation (P=0.000) whereas in control, difference was not significant (P=0.05). A negative correlation was observed between total thiol and lipid peroxidation (Figure 2), which shows that when oxidative stress is more, the total thiol molecule reduces due to oxidation (R^2=-0.369). Also a positive correlation (R^2=0.235) was observed between serum lipid peroxidation and number of cigarettes (Figure 3).

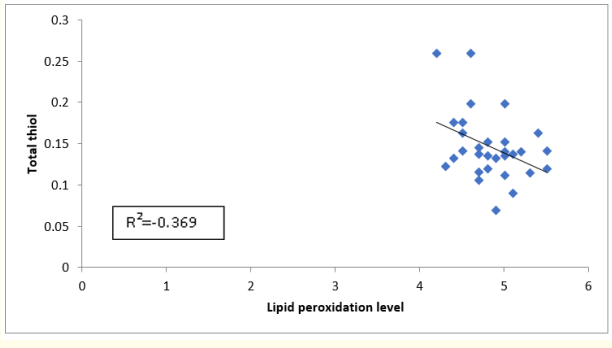

Figure 2: Correlation between serum lipid peroxidation and total thiol.

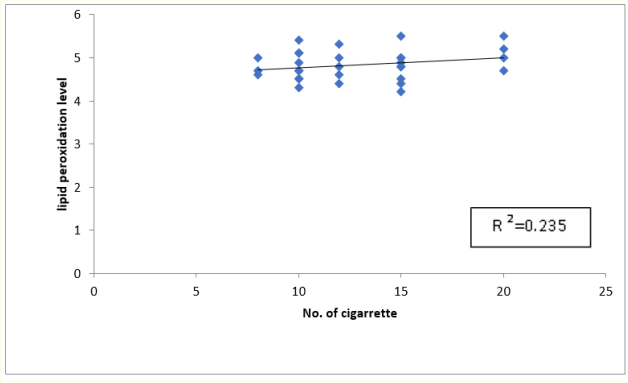

Figure 3: Correlation between serum lipid peroxidation and No. of cigarette per day.

Liu and Wei [27] reported that the level of total blood glutathione is negatively correlated with the level of plasma lipid peroxides (r= -0.305, P= 0.002) and was positively correlated with the smoking index (r=0.307, P=0.019) of all study subjects. These results indicate that the activities of glutathione peroxidase and glutathione S- transferase reduces to a great extent under smoking mediated oxidative stress in the blood of both young and ageing smokers. Moreover, the compensatory generation of total blood glutathione may effectively prevent plasma lipids from peroxidation in young smokers, although the activities of glutathione peroxidase and glutathione S-transferase in plasma were decreased. By contrast, total blood glutathione was inadequate for such protection in ageing smokers. It is suggested that supplementation of thiol group related agents may be considered for the prevention or alleviation of oxidative stress in ageing smokers, whose capability and capacity for the disposal of smoking mediated free radicals and reactive oxygen species are compromised.

Leelarungrayub., *et al.* [28] evaluated antioxidant potential of Thai shallot and reported that it protects human erythrocytes from possible damage from external or internal radicals such as H_2O_2 or peroxyl radical such as 2,2'-Azobis (2-amidino-propane) dihydrochloride. They reported that Thai shallot was able to inhibit lipid peroxidation and glutathione depletion in erythrocytes and sug-

gested that Thai shallot extracts have protective effect on the GSH deterioration *in vitro* from protein hydroperoxide or hydroxyl radical from gamma irradiation. Thai shallot extract can also protect and scavenge the protein and lipid hydroperoxide formation in *in vitro* study.

Total antioxidant by FRAP assay

It has been reported that smokers had 12% lower FRAP than non-smokers because of their increased oxidative stress. These data suggest that smokers have less plasma antioxidant potential, which would be consistent with their greater plasma isoprostane concentrations [29]. Plasma uric acid is the greatest predictor of FRAP and accounts for 60% of the total predicted FRAP, whereas ascorbic acid contributed to 15% of the value [11].

Results revealed that there was a significant increase in experimental group whereas changes in control group were not significant. Slightly positive correlation was observed between serum total antioxidant capacity and total thiol molecules (Figure 4). A negative correlation was seen between serum total antioxidant capacity and number of cigarettes smoked per day (Figure 5), whereas correlation between serum total antioxidant capacity and lipid peroxidation level was found to be positive (Figure 6).

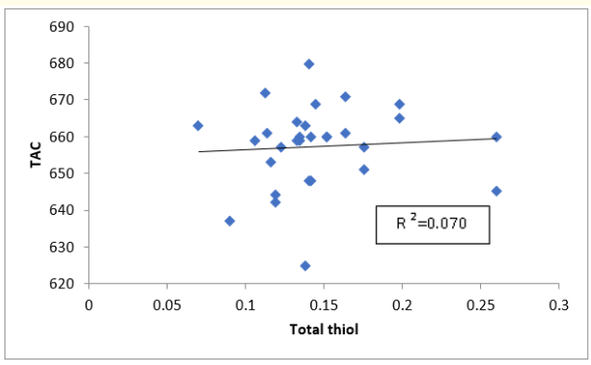

Figure 4: Correlation between serum total antioxidant capacity and total thiol molecule.

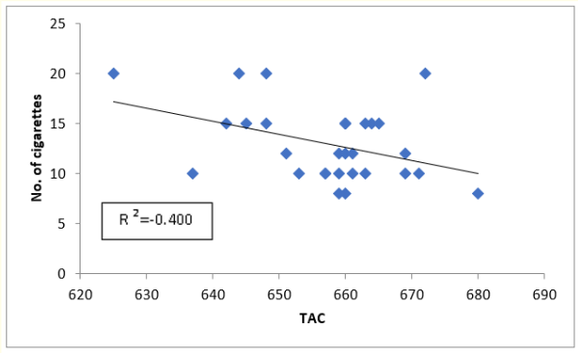

Figure 5: Correlation between serum total antioxidant capacity and No. of cigarettes per day.

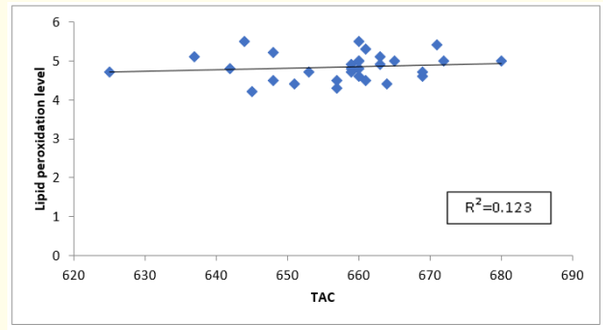

Figure 6: Correlation between serum total antioxidant capacity and lipid peroxidation level.

Conclusion

Since dry shallot demonstrated very high antioxidant activity in aqueous extract in our earlier study, the present research was planned to explore the antioxidant potential of dry shallot powder *in vivo* in a human trial in cigarette smokers. As seen by results, dry shallot powder was helpful in preventing oxidative stress in habitual cigarette smokers as judged by blood peroxidation levels. It can be suggested as a remedy to improve the health status of smokers.

Conflict of Statement

The authors have no conflict of interest in publishing this paper.

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Volume 3 Issue 7 July 2019