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Time Dependent Antioxidant Activity of Fresh Juice of Leaves of *Coriandrum sativum*

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ABSTRACT
The antioxidant activities of fresh juice of *Coriandrum sativum* was evaluated by various methods *in vivo*. Scavenging effects on the hydroxyl and super oxide radicals, level of glutathione and protection against reactive oxygen species induced lipid were evaluated. The fresh juice exhibited high antioxidant activities, evidenced by its ability to scavenge hydroxyl- and superoxide-radicals, high reducing power, and protection against biological macromolecular oxidative damage and by increasing the level of glutathione. Presence of flavonoids confirms its antioxidant activity. In conclusion, these results demonstrate potential antioxidant activity of fresh juice of *Coriandrum sativum*.

Keywords: *Coriandrum sativum*, antioxidant, flavonoids, glutathione, lipid peroxidation.

INTRODUCTION

Oxidation is one of the destructive processes, wherein it breaks down and damages various molecules. Oxygen via its transformation produces reactive oxygen species (ROS) such as super oxide, hydroxyl radicals, and hydrogen peroxide. [1] ROS are involved in a variety of physiological and pathological processes, including cellular signal transduction, cell proliferation, differentiation and apoptosis, as well as ischemia - reperfusion, inflammation, and many neurodegenerative disorders. [2] In healthy individuals, ROS production is continuously balanced by natural antioxidative defence systems. Oxidative stress is a process where the physiological balance between pro-oxidants and antioxidants is disrupted in favour of the former, ensuing in potential damage for the organism. [3] ROS production can induce DNA damage, protein carbonylation, and lipid peroxidation, leading to a variety of chronic health problems, such as cancer, aging, Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis. [4] Molecular oxygen is an essential component for all living organisms, but all aerobic species suffer from injury if exposed to concentration more than 21 %. [5] Free radicals attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins, and DNA.

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The body possesses several defense systems comprising enzymes and radical scavengers. Some of them constitute the repair systems for biomolecules that are damaged by the attack of free radicals. [6] Antioxidants are compounds that act as inhibitors of the oxidation process and are found to inhibit oxidant chain reactions at small concentrations and thereby eliminate the threat of pathological processes. [1] Phenolic compounds present in medicinal plants have been reported to possess powerful antioxidant activity. Flavonoids are known to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, neuroprotective, and anticarcinogenic activities. [7-8] Therefore, the search for natural antioxidants of plant origin has gained momentum in recent years.

The plant *Coriandrum savitum* (Dhania) belonging to the family *Apiacaea*. The major component in the oil is, S-(+) linolool. The other constituents isolated from the oil are flavonoids, quercetin, 3-glucoronide, monoterpene hydrocarbons viz., α-pinene, β-pinene, limonine, γ-terpinene, p-cymene etc. Borneol, citrollol, camphor, geraniol [9] and geranyl acetate. Coriander has been used extensively in folk medicine for its antimicrobial, antianxiety, analgesic, anticonvulsant, carminative, antifertility, antiasthamatic and insulin like activity. However its antioxidant action has not studied completely yet. Therefore, present study was undertaken to provide any experimental evidence.

MATERIAL AND METHODS

Plant material and preparation of fresh juice

C. sativum Linn. leaves were collected from herbal garden of Sree Siddaganga College of Pharmacy, Tumkur, Karnataka,

India. The plant sample was authenticated by Dr. S Siddapa, Head, Department of Botany, Sree Siddaganga College of Arts, commerce and science, Tumkur, Karnataka, India. A voucher sample specimen of the collected material was also deposited in the herbarium of Sree Siddaganga College. Leaves were separated from stem, washed properly in running water and juice was extracted by grinding the leaves into the mixer. The extract was filtered and used for the study.

Animals

Albino rats weighing 150-250 g of either sex were provided by animal house of Sree Siddaganga College of Pharmacy, Tumkur, Karnatka, India after approval of institutional ethical committee (Reg. No-SSCPT/IAEC-CLEAR/21/2004-05). The animals were housed in groups of six, in standard cages at room temperature in a 12:12 h light- dark cycle, with both food and water *ad libitum*.

Experimental Procedure

For antioxidant activity rats were randomly divided into five groups of six animals each. Animals in first group served as control and received vehicle, the second group received Vitamin C at the dose of 3 mg/kg body weight and used as standard. Groups III, IV and V received 2 ml of fresh juice of *C. sativum*. for the duration of 10, 20 and 30 days respectively. All the treatments were administered orally by gavage.

Determination of lipid peroxidation

Lipid peroxidation was measured by the thiobarbituric acid (TBA) reaction methodof Berton. In brief, samples were mixed with TBA reagent consisting of 0.375 % TBA and 15 % trichloroacetic acid in 0.25 N hydrochloric acid. The reaction mixtures were placed in boiling water bath for 30 min and centrifuged at 1811 g for 15 min. The absorbance of the supernatant was measured at 535 nm. MDA, a measure of lipid peroxidation, was calculated using an extinction coefficient of 1.56×10^5 /Mcm. The results were expressed as M/mg protein. [10]

Determination of antioxidant enzymes

Catalase activity

Catalase activity was measured according to the method of Aebi. One unit of catalase was defined as an amount of enzymes required to decompose 1µM of H_2O_2. The reaction was initiated by the addition of 1.0 ml of freshly prepared 20 nM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm for 1 min. The enzyme activity was expressed as U/mg protein. [11]

Super oxide dismutase activity

Estimation of SOD was done by autoxidation of hydroxylamine at pH 10.2, which was accompanied by reduction of NBT, and the nitrite produced in the presence of EDTA was detected colorimetrically. One enzymatic unit of SOD is the amount in the form of proteins present in 100 µl of 10 % liver homogenate required to inhibit the reduction of 24 mM NBT by 50 % and is expressed as units per milligram of protein. [12]

Glutathione

Glutathione was estimated using Ellman's reagent (5, 5¢-dithiobis-(2-nitrobenzoic acid) [DTNB]). The sulphydryl groups present in glutathione forms a colored complex with DTNB, which was measured colorimetrically at 412 nm. [5] The amount of glutathione was determined using its molar extinction coefficient of 13600/m/cm and expressed in terms of µmol/mg of protein. [13]

Statistical analysis

All the values are represented as mean ±SE (n=6). The statistical differences among different groups were analysed by one way ANOVA. The difference showing a p- level of 0.05 or lower was considered to be statistically significant.

RESULTS

Time dependent effect of fresh juice of *Coriandrum sativum* is shown in Table 1 and 2. At 2 ml/100 g body weight it had shown time dependent increase in Super oxide dismutase activity. In group I (control) was 11.38±0.005. In group III (10 days), group IV (20 days) and group V (30 days) was 17.76±0.004, 19.41±0.005 and 20.19±0.004 respectively. Vitamin C which is used as standard (group II) had Super oxide dismutase value 25.17±0.005 (Table 1).

We had also observed a significant ($p<0.05$) decrease in lipid peroxidation with increase in the time interval. The lipid peroxidation in group I was 2.87±0.06. In group III, group IV and group V was 2.19±0.03, 2.03±0.06 and 1.81±0.03 respectively, (Table 1).

The Catalase activity in group I (control) was 78.56±0.004 but group III, group IV and group V was 93.23±0.005, 129.59±0.004 and 138.27±0.004 respectively. In group II was 176.28±0.006. The Catalase activity increased with increase in the time interval, (Table 2).

As it seen from Table 2 there was significant ($p<0.001$) increase in Glutathione level in group I glutathione level was 6.79±0.05. In group III, group IV and group V glutathione level was 9.23±0.03, 11.69±0.02 and 12.19±0.05 respectively. In group II glutathione level was 16.23±0.05 (Table 2).

DISCUSSION

The present study was done for the investigation of antioxidant effect of fresh juice of *Coriandrum sativum*. The results obtained from the present study have been shown that fresh juice of *Coriandrum sativum* possesses antioxidant effect. It causes increase in Catalase, Glutathione and Super oxide dismutase activity where as decreases the lipid peroxidation. In the present study; Ascorbic acid used as a standard drug. Ascorbic acid is a water soluble antioxidant that maintains many cofactors in the reduced state. Ascorbic acid act directly on free radicals as well through interaction with vitamin E. [14-15] Ascorbic acid scavenge the super oxide radicals and thereby prevent free radical formation and lipid peroxidation. [16]

Lipid peroxidation is a complex process which is initiated by abstraction of hydrogen atoms from unsaturated fatty acids of phospholipids and lipoprotein complexes yielding conjugated dienes. The conjugated dienes then react with molecular oxygen to produce peroxy radicals which propagate the chain reaction by abstracting hydrogen from other unsaturated lipids. [17] Fresh juice of *Coriandrum sativum* lowers lipid peroxidation by maintaining the activities of antioxidant enzymes. *Coriandrum sativum* contain a chemical constituent called flavanoid. Flavonoids are a major class of phenolic compounds present to a great extent in many fruits and vegetables and also present in *Coriandrum sativum*. The basic flavanoid structure is a flavan nucleus, which consist of 15 carbon atoms in three rings. [18] Due to their lower redox potential flavonoids are thermodynamically able to reduce

Table: 1 Effect of Fresh juice of *Coriandrum sativum* on SOD and LP

S. No	Treatment	No of animals	Dose	Treatment Duration	SOD (unit/min/mg protein)	LP (n M/mg protein)
1	Control (saline)	6		10 days	11.38±0.005	2.87±0.06
2	Standard (VitaminC)	6	3mg/kg (0.3ml/100gm)	20 days	25.17±0.005**	1.13±0.04**
3	FJCS	6	2ml/100g	10 days	17.76±0.004	2.19±0.03
4	FJCS	6	2ml/100g	20 days	19.41±0.005	2.03±0.06
5	FJCS	6	2ml/100g	30 days	20.19±0.004*	1.81±0.03*

SOD-Super oxide dismutase, LP-Lipid peroxidase FJCS-fresh juice of *Coriandrum sativum ;* *P<0.05, **P<0.02

Table: 2 Effect of Fresh juice of *Coriandrum sativum* on catalase and Glutathione

S. No	Treatment	No of animals	Dose	Treatment Duration	Catalase (nM of H_2O_2/min/mg protein)	Glutathione (mol/ml)
1	Control (saline)	6	-	10 days	78.56±0.004	6.79±0.05
2	Standard (Vitamin C)	6	3 mg/kg (0.3ml/100gm)	20days	176.28±0.006**	16.23±0.05$^{\#\#}$
3	FJCS	6	2ml/100g	10days	93.23±0.005	9.23±0.03
4	FJCS	6	2ml/100g	20days	129.59±0.004	11.69±0.02
5	FJCS	6	2ml/100g	30days	138.27±0.004	12.19±0.05$^{\#}$

*P<0.05, **P<0.02, $^{\#}$P<0.01 $^{\#\#}$P<0.001, , FJCS-fresh juice of *Coriandrum sativum*

highly oxidizing free radicals by hydrogen atom donation. Thus the presence of flavonoid in a compound confirms its antioxidant activity.

Catalase is an enzyme present in the cells of plants, animals and aerobic (oxygen requiring) bacteria and an important member of the defense system against oxidative stress. It promotes the conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent, to water and molecular oxygen. [19] Catalase also uses hydrogen peroxide to oxidize toxins including phenols, formic acid, formaldehyde alcohols and detoxifying hydrogen peroxide and preventing the formation of carbon dioxide bubbles in the blood. The lowered activities of catalase shows the lack of lipid peroxide generation as lipid peroxides have been reported to induce catalase in vascular cells .In our present study the fresh juice of *Coriandrum sativum* increases the level of catalase .

Super oxide dismutase (SOD) is an enzyme that catalyzes dismutation of two superoxide anion (O^{2-}) into hydrogen peroxide and molecular oxygen .SOD is one of the most important enzymes in the front line of defense against oxidative stress. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady state level. [20] It is considered to serve as a cellular defense against the potentially harmful effects of superoxide anion generated by a wide variety of biological reactions. In the present study the increased SOD level indicates an elevated antioxidant status. [21]

Glutathione (g-glutamylcysteinylglycine, GSH) is a sulfhydryl (-SH) antioxidant, antitoxin, and enzyme cofactor. Glutathione is ubiquitous in animals, plants, and microorganisms, and being water soluble is found mainly in the cell cytosol and other aqueous phases of the living system. [22-23] It provides protection also for the mitochondria against endogenous oxygen radicals. Its high electron-donating capacity combined with its high intracellular concentration endows GSH with great reducing power. Glutathione is present inside cells mainly in its reduced (electron-rich, antioxidant) GSH form. In the disease state the activity of glutathione is decreases. In the present study the fresh juice of *Coriandrum sativum* increases the level of glutathione level which suggested that fresh juice of *Coriandrum sativum* decreases the level of free radicals in the body and potentiates the antioxidant activity. The study results showed that fresh juice of *Coriandrum sativum* has significant antioxidant activity.

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