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Oxidative stress in NIDDM patients: influence of coriander (*Coriandrum sativum*) seeds

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ABSTRACT

Oxidative stress is increased in diabetic patients since persistent hyperglycemia causes an increased production of oxygen free radicals through autoxidation of glucose and non-enzymatic glycation of proteins. Increased levels of the products of oxidative damage to lipids and proteins have been detected in the serum of diabetic patients. Administration of coriander seeds (5g/day) to NIDDM patients for 60 days countered oxidative stress as evidenced by significantly decreased lipid peroxidation, protein oxidation and decreased activity of erythrocyte catalase (CAT), increased serum β carotene, vitamin A, E and C in diabetics treated with coriander seeds. Besides, the treatment increased the activity of erythrocyte antioxidant enzyme i.e. glutathione–S–transferase (GST) and reduced glutathione content (GSH) in the treated diabetics. In conclusion, the treatment with coriander seeds ameliorated oxidative stress in diabetics due to the synergistic action of antioxidant phytochemicals, carotenoids, flavonoids etc. present in the seeds. From the findings of the study, the seeds are identified to possess antioxidant potential and hence, may be prescribed as adjunct to dietary therapy to combat oxidative stress in NIDDM patients.

Key words: Diabetes, Oxidative stress, Lipid peroxidation, Protein oxidation, Defense enzymes, Coriander seeds.

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INTRODUCTION

Diabetes is the world's largest endocrine disease with deranged carbohydrate, fat and protein metabolisms [1]. There is considerable evidence suggesting that oxidative stress plays a role in tissue damage associated with diabetes [2]. Oxidative stress is a condition in which the cellular production of reactive oxygen species (ROS: sometimes referred to as 'free radicals') exceeds the physiological capacity of the antioxidant defense system to render ROS inactivate [3]. Oxidative stress may be increased in diabetic patients since persistent hyperglycemia causes an increased production of oxygen free radicals (OFRs) through auto oxidation of glucose and non-enzymatic protein glycation [4]. Increased levels of the products of oxidative damage to lipids and protein have been detected in the serum of diabetic patients and their presence correlates with the development of complications [5]. Oxidants are counteracted by antioxidant enzyme systems such as catalase, superoxide dismutase (SOD) and glutathione peroxidase and by non-enzymatic antioxidant systems in the organism [6]. The efficiency of this defense mechanism is altered in diabetes [7].

The term antioxidant has been defined as "any substance exogenous or endogenous in nature that delays or inhibits oxidative damage to a target molecule[8] and protects biologically important molecules such as DNA, proteins, and lipids from oxidative damage and consequently reduce the risk of several chronic diseases" [9].

The traditional Indian diet, spices, fruits and vegetables are rich sources of natural antioxidants, called as "functional foods" provide more than simple nutrition; they supply additional physiological benefit. Spices with increased levels of essential vitamins and nutrients (e.g. vitamin E, lycopene, vitamin C etc.) provide a rich source of compounds like antioxidants including the flavonoids, thioredoxin, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthlalides [10].These antioxidants can be classified as enzymatic antioxidants: superoxide dismutase, catalase glutathione peroxidase, glutathione reductase and non enzymatic antioxidants like (nutrient antioxidants) β carotene, α–tocopherol, ascorbic acid, and metabolic antioxidants like glutathione [11]. Spices have also been recognized to possess several medicinal properties (diuretic, expectorant, laxative, antibacterial, antipyretic, etc.) and have been effectively used in India as well as in other countries [12]. Many spices and their active principles are excellent nutraceuticals [13]. Coriander (*Coriandrum sativum*) seeds possessing the nutritional as well as medicinal properties are among the most commonly used spices. Keeping in view of oxidative stress in diabetes and the medicinal properties of coriander seeds, the present investigation was undertaken to assess the influence of coriander seeds on oxidative stress in type 2 diabetes.

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MATERIALS AND METHODS

Procurement of seeds and preparation of powder

Coriander (*Coriandrum sativum*) seeds were procured from the local stores in Anantapur, thoroughly cleaned to free from extraneous matter and finely powdered using electric blender and placed in air tight containers. The powder was then packed in polythene covers to be used for clinical trial.

EXPERIMENTAL

Both male and female non insulin dependent (type 2) diabetes subjects in the age group of 40-60yrs. with no other specific complications were selected from local Diabetes Hospital on the basis of a specific questionnaire. Out of the selected subjects, 20 served as control and the other 20 served as experimental. The experimental group received coriander-seed powder 5g per day in 2 equal doses for a period of 60 days. All the subjects were given dietary guide lines and were under the supervision of a diabetologist.

Clinical analyses

At the initial and final stages of the experiment, fasting blood was drawn for the assay of various parameters. Fasting blood glucose [14], lipid peroxidation in plasma [15] and erythrocytes [16], protein oxidation [17], vitamin A and β-carotene [18], vit.C [19] and vit.E [20] in serum were estimated. Activities of catalase [21], glutathione–s–transferase [22] and reduced glutathione (GSH) [23] were assayed in erythrocytes.

Statistical analysis

Mean, standard error of means [24] and paired difference 't' test were conducted to assess significant difference between the data obtained before and after treatment.

RESULTS AND DISCUSSION

Fasting blood glucose levels in control subjects and experimental subjects treated with coriander seed powder were presented in **Fig.1** which indicated 11% ($p<0.001$) rise in control and 13% ($p<0.001$) decrease in coriander seed-treated type 2 diabetics. Hyperglycemia is the main risk factor for developing diabetic complications [25]. It is postulated that mitochondrial glucose overload results in increased electron transfer to oxygen and formation of free oxygen radicals. This in turn activates the pathways leading to diabetic complications along with hyperglycemia [26]. In hyperglycemia, there is enhanced metabolism of glucose through polyol (sorbitol) pathway, which also results in enhanced production of $^{\bullet}O_2^-$ [27]. Significantly more oxidative stress was reported in patients with type 2 diabetes than in healthy persons. Acute glucose fluctuations induce oxidative stress and these fluctuations were suggested to be

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valuable predictors for risk of diabetic complications [28]. Hence, in the present study, significant decrease in fasting blood glucose in coriander seed-treated group indicated control over hyperglycemia and decreased oxidative stress which is supported by significantly decreased lipid peroxidation (**Table 1**), a marker of oxidative stress in erythrocytes and plasma in coriander seed-treated group. The blood sugar lowering effect exhibited by coriander seeds is attributed to the phytochemicals viz. chlorogenic acid, pectin, protocatechuic acid and rutin, the micronutrients–ascorbic acid, niacin (vitamins) and minerals – chromium, copper, magnesium and zinc present in coriander seeds [29].

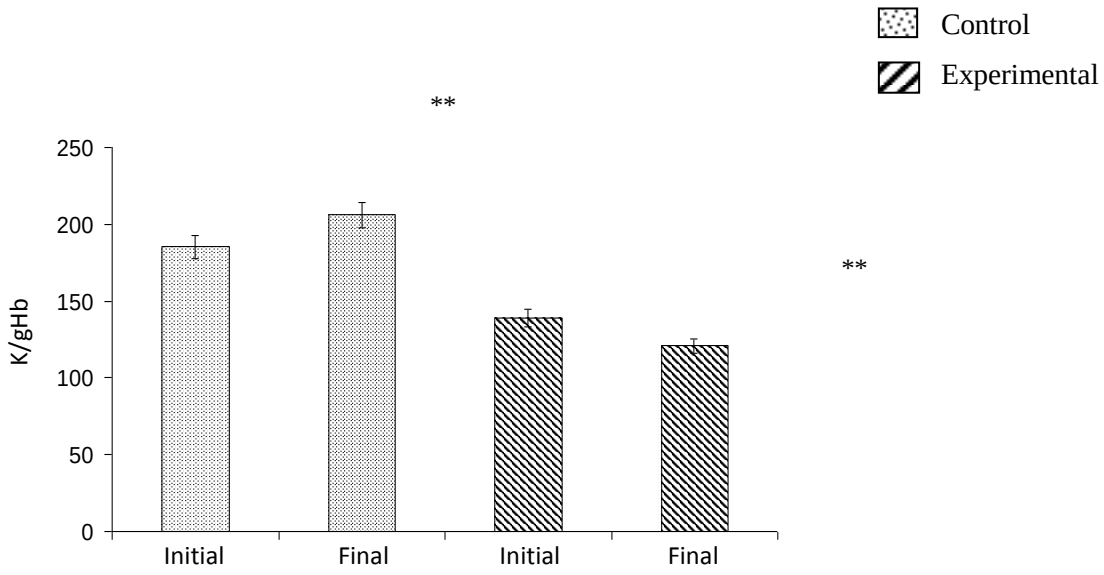

Fig.1 Fasting blood glucose in control and coriander-treated diabetics

Values are mean ± SEM of 20 subjects in each group
Comparison between initial and final; **p<0.001

Table-1: Effect of coriander seeds on lipid peroxidation and protein oxidation in experimental diabetics

Groups		Protein oxidation	Lipid peroxidation	
			Erythrocytes (nmol MDA/gHb)	Plasma (nmol MDA/dl)
Control	Initial	0.0051 ± 0.8	5.12 ± 0.6	419.81 ± 0.2
	Final	0.0054 ±1.0* (6)	10.09 ± 0.9** (97)	420.32 ± 3.0
Experimental	Initial	0.0060 ± 0.2	9.84 ± 0.9	419.01 ± 0.8
	Final	0.0030 ± 1.2** (50)	5.02 ± 0.5** (49)	317.33 ± 7.0** (24)

Values are mean ± SEM of 20 subjects in each group
The figures in parentheses indicate per cent increase /decrease over respective initial values.
Comparison between initial and final: * p<0.01; **p<0.001

Table 1 depicts the protein oxidation in serum, lipid peroxidation in erythrocytes and plasma in control and coriander seed-treated diabetes patients at initial and final stages of the experiment. The table indicates a 97% ($p<0.001$) increase in the erythrocyte lipid peroxidation in the control group. Treatment with coriander seeds decreased lipid peroxidation in erythrocytes by 49% ($p<0.001$), plasma by 24%, ($p<0.001$) as compared to the initial values in diabetes patients. Protein oxidation was decreased by 50 % ($p<0.001$) in coriander seed–treated group while was significantly increased (6%, $p<0.01$) in control group.

Lipid peroxidation is initiated by the attack on a fatty acid or fatty acyl side chain of any chemical species. Especially the group of polyunsaturated fatty acids (PUFAs) is highly susceptible to reactions with free radicals. Peroxidation of lipids in fatty acids may lead to a radical chain reaction. These chain reactions on substrate radical ($R^{\bullet}$) may result in the formation of many equivalents of lipid peroxides (LOOH) [30]. Diabetic red blood cells (RBCs) were shown to be more susceptible to lipid peroxidation as measured by TBARS in humans [31]. Similarly, increased plasma peroxide concentrations were reported in type 2 diabetes patients [32].

In the present study, decreased lipid peroxidation in the treated group in erythrocyte and plasma is attributed to the phytochemicals, radical scavengers present in the coriander seeds. As per the Duke's data base [29], the compounds possessing antioxidant activity in coriander seeds are the phytochemicals like apigenin, caffeic -acid, myristic acid, myristin, p-hydroxy-benzoic acid, palmitic acid, protocatechuic acid, isoquericitrin, gamma-terpinene, terpinen-4-ol, terpinolene, and trans-anethole. Most of these compounds act as radical scavengers, some of them reduce the radicals by donating hydrogen atoms, some of them also act as chain breaking agents in lipid peroxidation as a result of which a significant decrease in lipid peroxidation (a marker of oxidative stress) was brought about both in erythrocytes and plasma in treated subjects. Besides, the antioxidant property of the seeds was further evidenced by significantly decreased protein oxidation in serum (**Table 1**) in coriander seed-treated diabetics.

Proteins are an important target for oxidative challenge. Reactive oxygen species modify amino acid side chains of proteins to form protein carbonyls. Protein carbonyl content is the most widely used marker of oxidative modification of proteins and suggested to be a reliable marker of oxidative stress [33]. Elevated protein carbonyl stress was detected both in type1 and type 2 and also in experimental diabetes. Furthermore, the protein content is well correlated with the complications of diabetes [34]. A significant decrease in the protein oxidation along with significant decrease in lipid peroxidation in the present study, confirms control over oxidative stress in the treated diabetics.

Table 2 shows the levels of serum non enzymatic antioxidants (β-carotene, vitamin A, C, E) in type 2 diabetes patients at the initial and final stages of the experiment in control and coriander seed–treated group. Serum β-carotene and vitamin A levels were improved by 46% ($p<0.01$) and 38 % ($p<0.001$) respectively in coriander seed–treated group while the levels were decreased in the control group. This rise in serum β-carotene and vitamin A levels resulted in a

significant decrease in lipid peroxidation (**Table 1**) in erythrocytes and plasma in the treated group as carotenoids interact with free radicals that initiate harmful reactions such as lipid peroxidation. Carotenoids present in the sample with their highly reactive conjugated bonds act as free radical traps or antioxidants [35].

Table-2: Effect of coriander seeds on serum antioxidant vitamins in experimental diabetics

Groups		β Carotene (μg/dl)	Vitamin A (μg/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Control	Initial	166.2 ± 0.8	23.5 ± 0.8	1.93 ± 0.6	3.12 ± 0.2
	Final	141.6 ± 1.0 (15)	21.8 ± 1.0 (7)	1.88 ± 0.9 (2)	3.10 ± 1.0
Experimental					
	Initial	108.3 ± 0.2	20.6 ± 0.2	1.35 ± 0.9	2.98 ± 0.8
	Final	158.5 ± 1.2** (46)	28.5± 1.2** (38)	1.67± 0.5** (24)	3.64 ± 0.7** (22)

Values are mean ± SEM of 20 subjects in each group
The figures in parentheses indicate per cent increase /decrease over respective initial values.
Comparison between initial and final: * $p<0.01$; ** $p<0.001$

Especially β-carotene, a precursor of vitamin A, a nutritional antioxidant is known to protect membrane lipids from peroxidative damage. Its antioxidant ability is attributed mainly to the scavenging of several biologically damaging free radicals or reactive oxygen species such as singlet oxygen, peroxyl radical, superoxide and nitrogen dioxide [36]. The increase in free radical production with subsequent damage to the cellular processes observed in type 2 diabetes could be overcome with the supplementation of β-carotene as β-carotene traps free radicals and functions as an antioxidant [37]. Hence, raised serum β-carotene and vitamin A levels in the treated group lead to a decrease in the lipid peroxidation in the treated group.

In the present study, rise was observed in serum vitamin C levels i.e. 24%, ($p<0.001$) in coriander seed-treated diabetics whereas decrease was seen in controls. Ascorbic acid is a naturally occurring major antioxidant, essential for the scavenging of toxic free radicals, both in the plasma and tissues [38]. Previous studies suggest that oxidative stress is increased in diabetics and diabetic animal models [39]. The ascorbic acid levels in plasma and tissues of diabetes patients and animals have been reported to be low [40]. Supplementation of ascorbic acid decreased sorbitol levels [41] thereby preventing the development of diabetic complications [42] and increasing the stability of blood vessels [43]. In the present study, the raised ascorbic acid levels indicate decreased oxidative stress as ascorbic acid is a scavenger of the toxic free radicals generated in diabetes. This increase in vitamin C after treatment could also be due to protection of the existing vitamin C from oxidation to dehydro-ascorbic acid by some of the antioxidant phytochemicals present in the seeds under investigation.

The data presented in **Table 2** also indicates increase of 22 % ($p<0.01$) in serum vit. E in coriander seed–treated group compared to the initial values and a slight decrease was seen in

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control group. Rise in the serum vitamin C and E levels in type 2 diabetes in the present study might be because of the protection of the vitamins from oxidation because of the antioxidant phytochemicals and/or radical scavengers present in the seeds. Vitamin E is an important chain breaking and major lipid–soluble membranes bound antioxidant present in all the cells and protects the membranes from lipid peroxidation [44]. Its antioxidant efficiency is very high when considering its concentration in biological membranes which is very low [45]. It was shown that vitamin E regulates mitochondrial H_2O_2 generation and a high concentration of this vitamin reduces ROS production at mitochondrial level [46]. Both vitamin C and E quench free radicals by providing hydrogen atoms ($H^{\bullet}$) i.e., reducing equivalents that can pair up with unpaired electrons on the free radicals. In this process, vitamin E itself gets oxidized. The reduced forms of the vitamins are regenerated by ascorbate, NADH/NADPH and GSH can regenerate or spare each other so that vitamin C may spare vitamin E [47]. Therefore, in the present study, increased vitamin C (the quencher of free radicals) could prevent peroxidation of lipids (**Table 1**) in erythrocyte and plasma in type 2 diabetes.

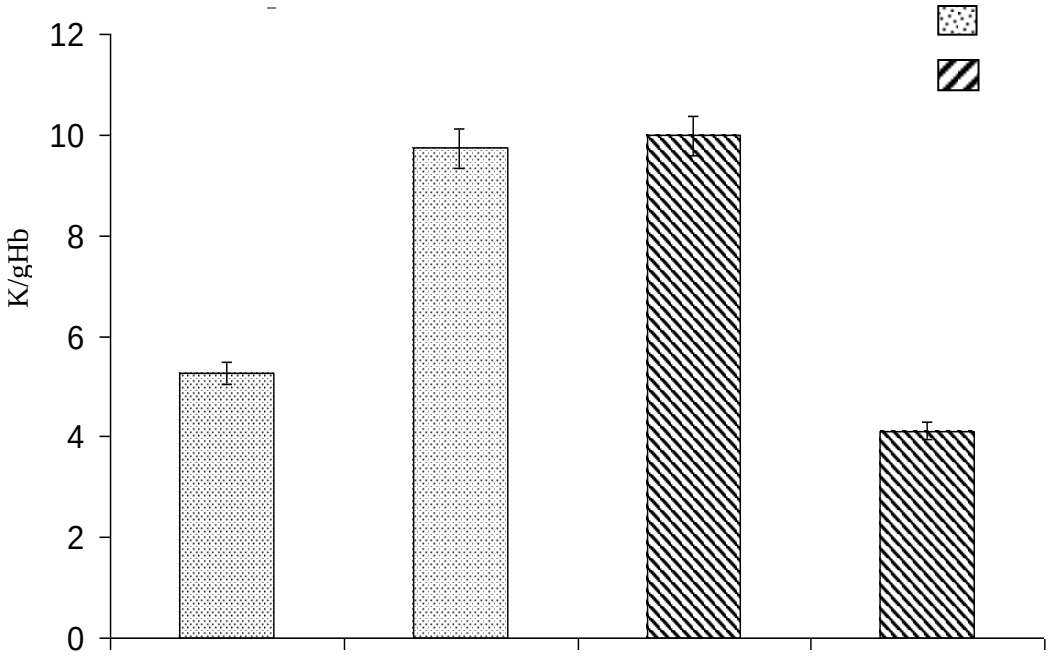

Fig.2 Activity of catalase in control and coriander-treated diabetics
Values are mean ± SEM of 20 subjects in each group
Comparison between initial and final; **p<0.001

Fig. 2-4 depicts the activity of erythrocyte enzymes and reduced glutathione. The data presented indicates a significant decrease i.e. 59% ($p<0.001$) in the activity of catalase in coriander seed-treated diabetics compared to the initial stage of the experiment. Catalase

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containing heme bound iron at its active site is a major primary antioxidant defense component that primarily works to catalyse the decomposition of H_2O_2 to H_2O and shares this function with glutathione peroxidase [48]. In the present study, a significant increase i.e. 86% ($p<0.01$) in the activity of catalase in the control group shows increased rate of radical production. Treatment with coriander seeds indicated a significant decrease in the activity of catalase, which indicates decreased H_2O_2 production as a result of the treatment given. This can be evidenced by the decreased lipid peroxidation (**Table 1**) in erythrocytes after the treatment.

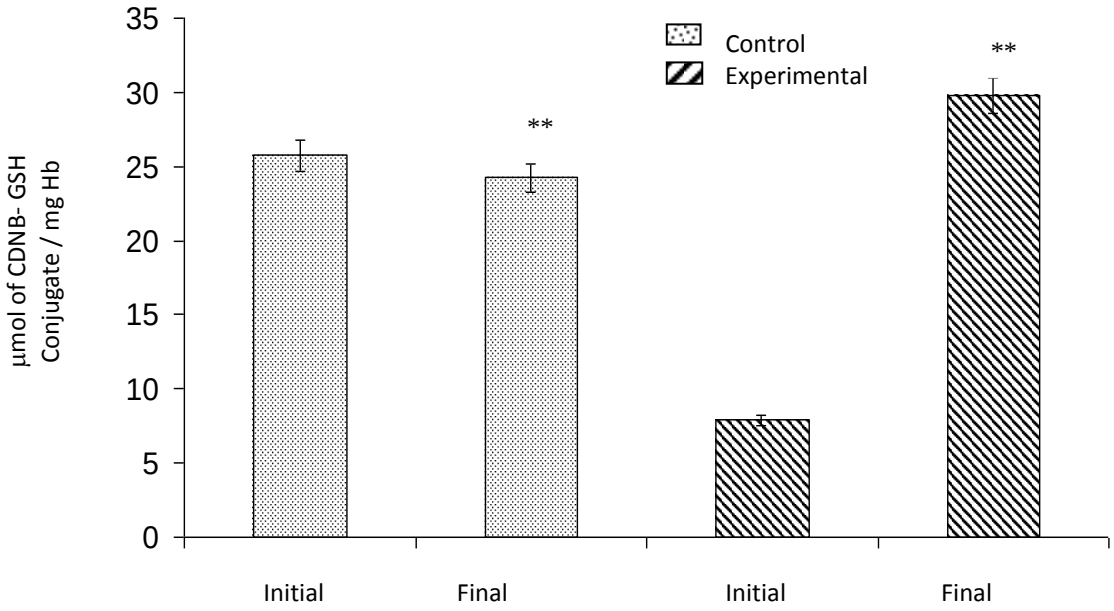

Fig.3 Activity of glutathione-s-transferase in control and coriander-treated diabetics
Values are mean ± SEM of 20 subjects in each group
Comparison between initial and final; **p<0.001

The data presented in **Fig 3** indicates a significant increase in the activity of GST (64%, $p<0.01$) in coriander seed-treated patients and a decrease of 22% in the control group. GST a multi functional protein found in many tissues plays an important role in the detoxification of xenobiotic compounds thereby protects the cell from peroxidative damage especially in the liver and also in lungs, tissues and erythrocytes [49]. Earlier reports indicated decreased activity of GST in liver and kidney of experimentally induced diabetic rats [50].Therefore, a significant increase in the levels of GST indicated protection against oxidative stress in the experimental group. This is further evidenced by remarkable increase in lipid peroxidation in RBC and plasma (**Table 1**) in controls that didn't receive any treatment and significantly decreased lipid peroxidation in coriander seed-treated group.

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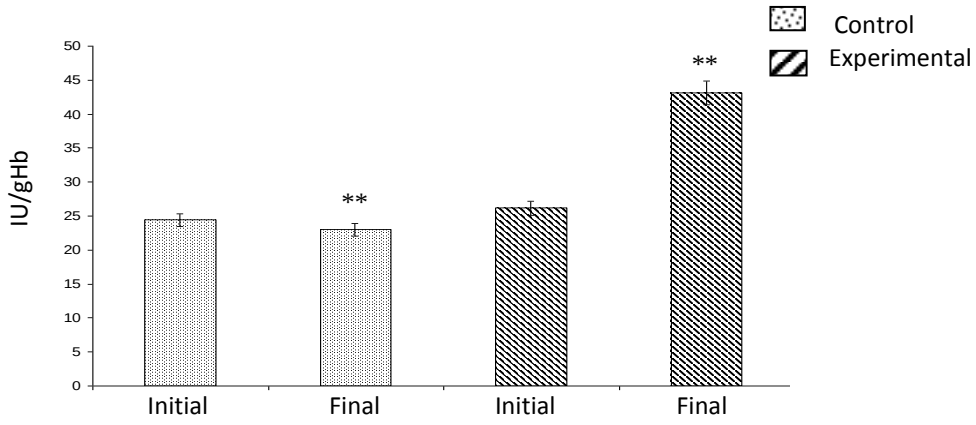

Fig.4 Reduced glutathione in control and coriander-treated diabetics
Values are mean ± SEM of 20 subjects in each group
Comparison between initial and final; **p<0.001

Fig.4 depicts the reduced glutathione (GSH) levels in the control and coriander seed-treated diabetes patients. Treatment with coriander seeds resulted in a tremendous and significant increase (278%, $p<0.001$) in erythrocyte GSH in diabetics. Glutathione participates in the detoxification at several levels and scavenge free radicals. Thus, glutathione provides the cell with multiple defenses not only against ROS but also against their toxic products. Marked alterations in antioxidant enzyme activities and tissue GSH concentration were reported in diabetes. Decreased GSH in diabetes may be caused by different pathways including 1) the increases sorbitol synthesis causing NADPH depletion and deficiency of this limits the reduction of GSSG to GSH catalyzed by glutathione reductase, 2) decreased activity of HMP shunt enzymes which generate NADPH and 3) transport of GSSG through erythrocyte membranes due to oxidative stress induced membrane damage[51]. In poorly controlled diabetic condition, impaired glutathione system by inactivation of GPx and GR may contribute to the initiation and / or progression of diabetic complications [52-53]. Hence, significantly increased GSH in the treated group in the present investigation indicates control over oxidative stress. This is further supported by significant decrease in lipid peroxidation (**Table 1**) in erythrocytes and plasma of the treated subjects and increased serum vit.C and E as GSH regenerates these vitamins.

CONCLUSIONS

In-vivo experiments revealed antioxidant role of coriander seeds which is evidenced by elevated serum non-enzymatic and erythrocyte enzymatic antioxidants and very effectively decreased lipid peroxidation in erythrocytes and plasma in type 2 diabetes patients. Further investigations on the mechanism of action of active principles in coriander seeds are in progress.

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