

Effect of Turmeric and Carrot Seed Extracts on Serum Liver Biomarkers and Hepatic Lipid Peroxidation, Antioxidant Enzymes and Total Antioxidant Status in Rats

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ABSTRACT

Introduction: Pathogenic role of free radicals are well known in various metabolic diseases. They originate from internal and external sources of body. Essential roles of antioxidant defense system for cellular redox regulation and free radical scavenging activity were described in this study. Many *in vitro* investigations have shown that turmeric (TE) and carrot seed extract (CSE) exhibits to possess antioxidant activities. In this study, we evaluated the antioxidant potentials of ethanolic TE and CSE based on *in vivo* experiment in the rats. **Methods:** Animals were assigned to six groups: the 1st and 2nd groups were control groups and 2nd group received 0.2 ml dimethyl sulphoxide as vehicle treated group; other four experimental groups received different doses of TE (100, 200 mg/kg b.w.) and CSE (200, 400 mg/kg b.w.) by gavages, respectively for a period of one month. The indicators of oxidative stress, lipids peroxidation, markers of hepatocyte injury and biliary function markers were measured. **Results:** The levels of superoxide dismutase, catalase, and glutathione peroxidase were significantly stimulated in the hepatic tissue of treatment groups. The malondialdehyde contents of liver tissue were significantly reduced in the groups fed with TE and CSE. Serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, in treated groups were found to be significantly decreased, whereas albumin and total protein increased as compared to the control groups ($P < 0.05$). **Conclusion:** this study showed that the regular intake of TE and CSE through the diet can improve antioxidant status and inhibit peroxidation activity in the liver tissue so that using these extracts may protect tissue oxidative stress.

Introduction

There are numerous reports showing that uncontrolled and excessive production of reactive oxygen species (ROS) can have devastating consequences for cells. ROS are continuously generated in all living cells in metabolism process. Respiration as a kind of metabolic process determined by the use of oxygen is the being-reason of these materials. It is explored that almost 100 diseases have been recognized by ROS acts (Sies 1991). ROS include free radicals such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals (OH^{\cdot}), alkoxy radical (RO^{\cdot}) and

peroxyl radical (ROO^{\cdot}) and non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2) (Gülçin 2006, Halliwell *et al* 1998). Moreover, reactive nitrogen species (RNS) play important biological role. Nitric oxide (NO) and peroxynitrite are components of RNS with main biological activities. Both of ROS and RNS are generated spontaneously under physiological conditions. Undesirable byproducts of these species can easily affect the peroxidation membrane in lipids and intensify nucleic acid and proteins, and damage carbohydrates (Halliwell *et al* 1998).

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The defense against these species by all aerobic organisms is performed by the antioxidant acts, antioxidant enzymes and antioxidant food constituents. In both synthetic and natural antioxidants, the diseases and health problems can be depleted by reducing the rate of free radicals and increasing the defense factors, (Ames *et al* 1993). Healthy individuals are balanced in ROS/RNS production and antioxidant defenses. "Oxidative stress" is induced by imbalance occurrence between free radical reaction and activity of the antioxidant defenses (Byun *et al* 1999).

Recently there has been a dynamic research and interest in exploiting natural compounds that proportionately advocate the body from any disease which is caused by ROS. These defensive materials lead to free radical formations' reduction and ultimately the oxidative stress (Kushi *et al* 1996, Halliwell *et al* 1990). The prohibiting act against ROS intrusively rejects the other forms of disease which might have occurred by the ROS, namely the diabetes, cancer, and atherosclerosis that are resulted from lipid peroxidation in tissue (Halliwell *et al* 1989).

The spices and plants have widely been used in foods since ancient times to engender good odor, flavor, color and preservative. Nowadays, it is a clear fact that they possess antioxidant activity and the reason for decreasing oxidation potential of lipids in foodstuffs. Meanwhile, ten-thousands of them have been employed as a natural defense against diseases and infections (Abad *et al* 2011a, 2011b). Now, it is most common to explore the antioxidant activity of these compounds (Kikusaki *et al* 1993, Yamana and Balicki 2010, Sharma and Shukla 2011). The current study is limited to TE and CSE, two natural products which are in use for many years.

TE is an active principle of the perennial herb *Curcuma longa*. Its antioxidant property as well as therapeutic properties against various diseases like cancer, hepatitis and inflammatory diseases have been reported (Goel *et al* 2008, Bengmark 2006). It is charismatic spice, since its acts in scavenging the free radical are strikingly considered. Currently, it has attracted much attention due to its significant medicinal potential (Aggarwal *et al* 2003). TE is an effective antioxidant in preventing lipid oxidation (Shalini and Srinivas 1997).

Daucus carota commonly known as "Carrot" is one of the most important vegetables and belongs to the family of Apiaceae. Its active ingredients like volatile oils, steroids, tannins, flavonoids, and carotene have been isolated (Mahran *et al* 1991, Vasudevan *et al* 2006, Jasicka-Misiak *et al* 2005). Carrot seeds are rich of antioxidants (Yu *et al* 2005). On the other hand, carrot contains carotenoids which are natural pigments with lipophilic properties and antioxidant characteristic. The major carotenoids in human plasma are β -Carotene and lycopene. They are transported in blood complexes to

plasma lipoproteins, mainly to the LDL particle. LDL particle supplements with β -carotene or with lycopene and renders an increased resistance to oxidation (Fuhrman *et al* 2000, Sesso 2006). The higher serum carotenoid concentrations, the lower risk of diabetes and insulin resistance can be caused by carotenoids function (Hozawa *et al* 2006).

The data obtained from *in vitro* assessment have encouraged us to conduct an investigation and compare antioxidant properties of TE and CSE in liver tissue of rats.

Materials and methods

Chemicals and reagents

All chemical kits were Randox[®] kits (Cat No. NX2332, Randox laboratories, Ltd, Crumlin, UK). Dimethyl sulphoxide (DMSO), ethylene diamine tetracetic acid (EDTA) 3-(4,5-dimethylthiazol-2-yl), ethanol, ethyl acetate, Tris HCl, 2-thiobarbituric acid (TBA) and other chemicals were obtained from Sigma Chemical Co. (St Louis, MO, USA). All chemicals used were of analytical grade.

Preparation of the ethanolic TE and CSE

Curcuma longa and *Daucus carota* were purchased from local market and identified by the Cultivation and Development Department of Institute of Medical Plants, Tehran, Iran. Extracts' preparation was done according to the method presented by Santosh *et al* (1996). Both plants were thoroughly washed, air dried and pulverized in a grinding mill to a perfect powder. Four hundred grams of each plant were weighted and macerated with 90% ethanol. The mixture was frequently filtered and centrifuged. The supernatant was evaporated by employing rotary under reduced pressure at 40°C. Finally, the extracts were dissolved in DMSO (0.2 ml, 50% v/v) at different concentrations for TE (100, 200 mg) and CSE (200, 400 mg).

Animals

Adult male Wistar strain rats (weighing between 220-280 g) were used for this experiment. The rats were kept in the special animal rooms, housed in cleaned and disinfected typical plastic cages under 12h light and 12h dark cycle with free access to the water during the investigation.

Experimental design

42 male Wistar rats were randomly divided into six groups including seven rats per group. Groups one and two were controls. Group 2 received 0.2 ml DMSO by gavages as vehicle; other four experimental groups received different doses of TE (100, 200 mg/kg b.w.) and CSE (200, 400 mg/kg b.w.) by gavages, respectively. The experiments were administered during 8-10 a.m. and total period of study was one month. At the end of experiment, blood samples were collected

from the retro-orbital plexus and processed to obtain serum for the determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Albumin (ALB), Total protein (TP), and plasma total antioxidant status (TAS). In order to obtain liver tissues, all animals were sacrificed and sampled. Obtained tissues immediately were frozen in liquid nitrogen and stored at -70°C until laboratory tests were done. The tissues were homogenized in 1.15% KCl solution and 20% (W/V) homogenate was prepared.

Measurement of antioxidant status

Measurement of TAS level

Collected blood samples were separated following centrifugation at 4000 rpm for 10 min. Therefore, plasma TAS was measured using a novel automated colorimetric version of the total antioxidant response (TAR) method (Erel 2004).

Measurements of superoxide dismutase (SOD)

For measuring SOD activity, the homogenized liver tissues were assayed by a spectrophotometric method based on the inhibition of a superoxide-induced reduced nicotinamide adenine dinucleotide (NADH) oxidation according to Paoletti *et al* (1986).

Glutathione peroxidase (GPx) activities

GPx activity was estimated in liver tissues according to Paglia and Valentine (1967).

Catalase (CAT) activities

Method presented by Claiborne (1985) was used for CAT measurement.

Estimation of lipid peroxidation

Malondialdehyde (MDA) levels were assessed utilizing the thiobarbituric acid reactive substances (TBARS) method (Kaya *et al* 2004).

Biochemical analyses

Plasma was used to measure serum ALT and AST as indicative of hepatic function. ALP was measured to assess biliary function and ALB was measured to reflect liver synthetic function. The plasma AST, ALT, ALP, ALB, and TP activities were estimated using commercially available kits by autoanalyzer.

Statistical analysis

The results were analyzed using SPSS 17. The descriptive statistics of mean \pm SD were stated and statistical comparison among groups were performed by one way analysis of variance (ANOVA) followed by Tukey's test. $p < 0.05$ was considered as statistical significance.

Results

The activities of SOD, GPx and CAT changes in liver are shown in Table 1. In TE and CSE400 treated groups, the significantly elevated liver SOD level was observed compared to the control groups that did not received TE and CSE ($p < 0.05$). The level of SOD in TE200 was

significantly increased compared to control group ($p < 0.01$) and significantly increased with respect to CSE200 ($p < 0.05$). As can be seen in this table, GPx content was significantly increased in TE200 ($p < 0.05$) and CAT level in groups TE100, TE200 and CSE400 were significantly boosted compared to control groups ($p < 0.05$). However, TE200 dramatically increased in comparison with control group ($p < 0.05$).

Table 1. Effect of turmeric and carrot seed extracts on antioxidant status and lipid peroxidation in rat liver

Groups	SOD (U/ml/tissue)	GPx (U/ml/tissue)	CAT (U/mg protein)
Control	4.7 \pm 0.34	3.9 \pm 0.28	2.37 \pm 0.25
DMSO	4.4 \pm 0.23	3.7 \pm 0.39	2.13 \pm 0.10
TE(100mgkg ⁻¹)	5.7 \pm 0.27 ^a	4.1 \pm 0.44	3.14 \pm 0.17 ^a
CSE(200mgkg ⁻¹)	6.1 \pm 0.39 ^{b,c}	4.2 \pm 0.31 ^a	3.94 \pm 0.38 ^{b,c}
CSE(200mgkg ⁻¹)	4.9 \pm 0.34	4.1 \pm 0.01	2.70 \pm 0.24
CSE(400mgkg ⁻¹)	5.6 \pm 0.40 ^a	4.1 \pm 0.50	3.30 \pm 0.13 ^a

DMSO: Dimethyl sulphoxide; TE: turmeric; CSE: Carrot seed extract; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; CAT: Catalase.

a: significantly different from control at $p < 0.05$; b: significantly different from control at $p < 0.01$; c: significantly different from CSE200 at $p < 0.05$.

According to the results shown in Fig. 1, MDA level in liver tissue was lower in treated groups than that in control groups. Also, there is no significant decrease in CSE200 ($p > 0.05$). It is also clear from Fig. 2 that, TAS activity in plasma was found to be significant ($p < 0.05$) in groups TE100, TE200 and CSE400. Meantime, TAS level was increased significantly in group TE200 in comparison with CSE200 ($p < 0.05$). Effect of TE and CSE on biochemical changes in rat liver was shown in Table 2. ALT, AST, ALP serum contents in TE200 were found to be significantly decreased in compared comparison with Group 1, while ALB and TP levels were found to be significantly decreased.

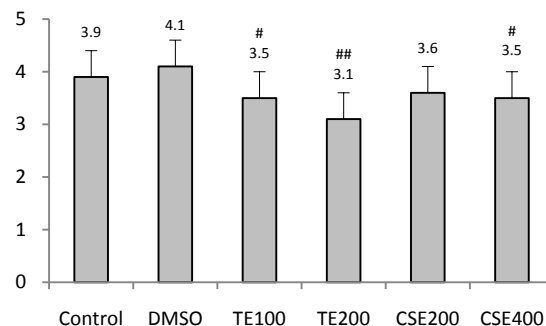


Fig. 1. Effect of turmeric and carrot seed extracts on plasma MDA (nMol/ml) in rat liver. TE200 treatment significantly reduced MDA level ($P < 0.01$), and TE100 and CSE400 significantly decreased MDA level ($P < 0.05$) in comparison with control group. #: $P < 0.05$; ##: $P < 0.01$.

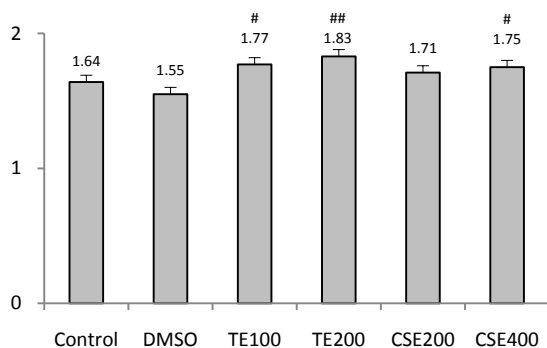


Fig. 2. Effect of turmeric and carrot seed extracts on plasma TAS (mmol Trolox Eq/L) in rat liver. TE200 treatment significantly increased TAS content ($P<0.01$), and TE100 and CSE400 significantly increased TAS content ($P<0.05$) in comparison with control group. #: $P<0.05$; ##: $P<0.01$.

Table 2. Effect of turmeric and carrot seed extracts on serum liver biomarkers in rat

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	ALB (g/dL)	TP (g/dL)
Control	166.3±5.0	46.60±2.60	190.87±7.20	4.3±0.50	8.6±0.11
DMSO	168±6.30	47.91±2.40	192.44±5.78	4.2±0.76	8.4±0.42
TE(100mgkg ⁻¹)	158±4.51 ^a	44.30±2.77	188.44±7.45	4.4±0.21	9.1±0.15 ^a
TE(200mgkg ⁻¹)	153±4.98 ^b	42.88±4.10 ^a	185.44±5.78 ^a	4.9±0.81 ^a	9.7±0.33 ^{a,c}
CSE(200mgkg ⁻¹)	163±5.66	46.23±2.21	189.44±6.45	4.3±0.44	8.5±0.65
CSE(400mgkg ⁻¹)	160±5.78 ^a	44.71±3.00	187.44±8.21	4.5±0.10	8.9±0.34

DMSO: Dimethyl sulphoxide; TE: Turmeric; CSE: Carrot seed extract; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; ALB: Albumin; TP: Total protein.

a: significantly different from control at $p<0.05$; b: significantly different from control at $p<0.01$; c: significantly different from CSE200 at $p<0.05$.

Discussion

Oxidative defensive system is able to remove, neutralize, or scavenge oxyradicals and their multitude of reactive oxygen species-producing contaminants. Parts of this defense system can be affected by nutrients and non-nutrients. Data suggest that natural products can exert influence on important components of enzymatic antioxidative defenses such as SOD and GPx. It is well-known that activities of these enzymes modulate oxidative damage within tissues due to attenuation of oxidative stress. Our result showed that TAS level increased in treated groups fed with TE and CSE, however, in group TE200 the level of TAS were high in comparison with other groups. This process can be quantitated in terms of lipid peroxidation and protein oxidation (Kota *et al* 2008, Kuo *et al* 2009). It is also well documented that lipid peroxidation is as one of the molecular mechanisms augmented during high activity, toxicity induced and in some diseases (Berzosa *et al*

2011, Kehrer 1993, Nourooz-Zadeh *et al* 1997). These findings are in line with our results. The ROS and Free radicals scavenging capacity is elevated by antioxidants from deleterious effects. Plasma TAS is a combination of different antioxidant defensive system, including enzymatic and nonenzymatic systems. Elevation of plasma antioxidant capacity can attenuate development of lipid peroxidation (Ohkawa *et al* 1979).

Extensive research within the past half-century has indicated that *Curcuma longa* is a nutraceutical compound reported to possess therapeutic properties in order to prevent a broad range of diseases (Liu *et al* 1997). In addition, it exhibits antioxidant, anti-inflammatory, and proapoptotic activities (Sandur *et al* 2007). The results of their study are also in line with our findings.

Meanwhile, there are number of *in vitro* assays were found that curcumin has antioxidant activities like DPPH[•], ABTS^{•+}, O₂^{•-} and DMPD^{•+} radical scavenging, hydrogen peroxide scavenging and metal chelating activities. It has been proved that TE and curcumin can be used for minimizing or preventing lipid oxidation in pharmaceutical product retarding the formation of toxic oxidation products, maintaining nutritional quality and prolonging the shelf life of pharmaceuticals (Tuba *et al* 2008, Ahmida 2012). Present study showed significant reduction in MDA content in liver tissue of plant fed groups. It suggests that the antioxidant status of host tissue can be enhanced by consuming TE and CSE through the diet.

Fatty compound profiles, antioxidant properties and other constituents of carrot seed recently have been considered by Yu (2002) and Perret *et al* (2004). They suggest that more than 80% of fatty compounds of carrot seed are including oleic acid. However, oxyradical scavenging capacity of carrot seeds were found 160 μmolTE/g as well as polyphenolic compounds and radicals neutralize capacity of this plant were reported 1.98 mgGE/g and 8.9 μmolTE/g, respectively (Yu *et al* 2002, Parry *et al* 2004). Several *in vivo* investigations showed that CSE might have anti-fertility property (Bhatnagar *et al* 1995, Majumdar *et al* 1998), cholinergic activity (Gambhir *et al* 1966), analgesic and anti-inflammatory activities (Vasudevan *et al* 2006). Other studies indicated that root extract of this plant has been found to have hypoglycemic and hepatoprotective properties (Neef *et al* 1995, Bishayee *et al* 1995). Our study showed that relevant effects of TE and CSE against cited complicates which are associated with oxidative stress and inflammation may be due to their antioxidant properties when administered orally.

In present study, we showed that administration of TE and CSE significantly increased hepatic tissue concentrations of SOD, GPX and CAT in treatment groups. This result is consistent with the findings of

several reports (Sandur *et al* 2007, Yu *et al* 2002, Parry and Yu 2004). Main three antioxidant enzymes cooperate together as mutually supportive team to detoxify the cells. SOD could change free radical superoxide to peroxide and GPX or catalase can attack and destroy the peroxide. SOD converts superoxide to hydrogen peroxide and molecular oxygen. Another function of SOD as a first antioxidant enzyme is to protect the dihydroxy acid dehydrates, aconitase, 6-phosphogluconate dehydratase and fumarases A and B against inactivation by the free radical superoxide (Benov and Fridovich 1998). SOD converts superoxide to hydrogen peroxide and molecular oxygen. Catalase is one of the most efficient enzymes known which never can be saturated by H₂O₂ at any concentration (Lledías *et al* 1998). CAT could decompose H₂O₂ to water and oxygen. GPx catalyses cause the reduction of product of a variety of hydroperoxides (ROOH and H₂O₂) and reduced glutathione to form glutathione disulphide (GSSG) (Mates *et al* 2006). Our study revealed that administration of TE200 to the rats could significantly increase antioxidant properties such as boosting of SOD, GPx, CAT and decreasing MDA rates in comparison with other treated groups (TE100, CSE200 and CSE400). Also, the hepatic level of TAS was normalized with TE and CSE; however, better result was observed at the dose of 200 mg/kg of TE. Measurement of total antioxidative response reflects the redox status of plasma which shows the antioxidant components in plasma (Erel 2004). According to the literature, the plasma TAS level in some diseases is low e.g. in cancer and rheumatoid arthritis (Altindag *et al* 2007).

The most common LFTs include serum aminotransferases, alkaline phosphatase and ALB. In the current study, significant decline in markers of liver injury (ALT, AST and ALP) reflects modulating hepatocytes safety in treated groups. The obtained results of our study displayed the effectiveness of TE in normalization of the liver enzymes AST and ALT, and liver function tests like ALB and total proteins. In addition, the best results were found with TE at the dose of 200 mg/kg b.w. (Table 1 and 2). It should be pointed out that depending on the dose of TE and CSE, antioxidant enzymes and redox state were regulated. Liver function tests (LFTs) are commonly employed in clinical practice related to screen for liver disease. Hepatocellular damage results in release of these enzymes into blood circulation. Increase in serum levels of AST shows hepatic injuries similar to viral hepatitis, infarction and muscular damages. ALT, which mediates conversion of alanine to pyruvate and glutamate, is specific for liver and is a suitable indicator of hepatic injuries. Increased levels of these enzymes are indicators of cellular infiltration and functional disturbance of liver cell membranes (Drotman and Lawhan 1978). In addition, ALP is membrane bound and its alteration is

likely to affect the membrane permeability and produce derangement in the transport of metabolites (Mehana *et al* 2010). On the other hand, bilirubin and ALB values are associated with the function of hepatic cells (Muriel *et al* 1992). The data of our study also revealed that daily treatment of TE and CSE extract markedly regulated biochemical status of rats.

Conclusion

The data by this study showed that orally administrations of TE and CSE could enhance the levels of antioxidant enzymes and subsequently would reduce the oxidative damage of liver tissues in rats. Antioxidant activity was boosted due to an increase in the above-mentioned doses of plants. All studied parameters showed that treated rats with TE and CSE for a month period could have protective effect in hepatic tissue. However, TE200 was found to have significant difference in comparison to other treated groups.

Ethical issues

We followed ethical guidelines approved by the Institutional Animal Ethics Committee located in Islamic Azad University and laboratory conditions were provided according to university guidelines for caring.

Conflict of interests

Authors declare no conflict of interests.

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