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## Effect of Dietary Supplementation of *Ellataria cardamomum* and *Nigella sativa* on the Toxicity of Rancid Corn Oil in Rats

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**Abstract:** In the present study *Nigella sativa* and *Ellataria cardamomum* seeds have been studied as inhibitors of oxidative stress caused by oxidized corn oil (having PV 389.8 meq kg<sup>-1</sup>) in rats. The 70 days feeding male albino rats with experimental diets did not produce significant changes in the body weights, organ weights and food intake of different groups. Increased lipooxidative damage was noticed in oxidized oil fed rats. Oxidized oil diet supplemented with cardamom or *N. sativa* had marked reduction in RBC hemolysis and plasma AST/ALT activity. The formation of thiobarbituric acid reactive substances were lowest in rats fed *N. sativa* supplemented diet. Reduced glutathione of liver and kidney were significantly raised after the addition of cardamom/*N. sativa* to the diets compared to oxidized oil fed rats (Group F). But heart reduced glutathione showed a significant increase as compared to oxidized oil fed group only after the supplementation of *N. sativa* to the diet. These data indicates that *N. sativa* or cardamom supplementation improves the overall antioxidant protection capacity of the body.

**Key words:** Cardamom, *Nigella sativa*, antioxidants, rats, ALT, hemolysis, TBARS

### INTRODUCTION

Increased risk of some chronic heart diseases especially cardiovascular disease and certain types of cancers are associated with high intake of fat<sup>[1]</sup>. In developed countries the fat intake is relatively high, due to frequent consumption of fried food. Thermally oxidized fat is generally considered to contain potentially toxic lipid peroxidation products<sup>[2]</sup>.

Many antioxidants are found in spices and herbs and they may be effective to protect against the peroxidative damage caused in living cells<sup>[3,4]</sup>. The spices and spice principles are also known to possess anticarcinogenic and anti-inflammatory properties<sup>[5,6]</sup>. These beneficial effects are mediated in part by inhibiting the formation of lipid peroxides<sup>[7]</sup> and prostaglandin synthesis<sup>[6]</sup>. Spices also aid in digestion by intensifying the salivary flow and gastric juice secretion<sup>[8]</sup>.

Antioxidants are not only the principle ingredients in food that protect foods by inhibiting oxidative breakdown of their lipid component. But they are also important for the defense of living cells against oxidative damage. Phenolics found in most of the seeds retards lipid peroxidation in native seed as well as in the extracted oil. They may acts as primary antioxidants, synergists

and chelators. Many different kinds of antioxidants are found in various plants<sup>[9,10]</sup>. The *Nigella sativa* is a herbal medicine, used for the treatment of variety of ailments<sup>[11]</sup> and also commonly used as condiment in Middle East. It has also been reported to possess many pharmacological effects. Thymoquinone an active principle of *N. sativa* seed is known to possess antioxidant property<sup>[12]</sup>. The fruit of cardamom, *Ellataria cardamomum* is usually used as a flavouring agent in Arabian coffee throughout the Arabian countries and as spices in many countries. They are medicinally used for flatulent indigestion and to stimulate the appetite in people with anorexia. The seeds of cardamom were also prescribed in Ayurvedic medicine for cough, cold, asthma, indigestion and bronchitis<sup>[13]</sup>. Eugenol an active principle of cardamom<sup>[14]</sup> is also known for its antioxidant property<sup>[3]</sup>.

The overall goal of the study was to assess the relative efficacy of *N. sativa* and cardamom seeds as modulators of oxidative stress induced by feeding highly rancid oil to rat.

### MATERIALS AND METHODS

Thiobarbituric acid was the product of Fluka Switzerland. *N. sativa*, cardamom and corn oil were

purchased from local supermarket. *N. sativa* and cardamom were cleaned and powdered. 1,1,3,3-Tetraethoxypropane (TMP) was obtained from Sigma (St. Louis, Mo, USA). All other chemicals were of reagent grade and used without further purification.

**Preparation of oxidized corn oil:** Fresh corn oil was purchased from local supermarket. The oil was subjected to the following oxidation process: 2 kg corn oil was taken in a glass beaker and aerated in a water bath at 80°C. The oxidation proceeded for 8 h/day and was repeated successively for 40 days. The resultant oxidized oil was stored at -20°C until the test diets were prepared. The fatty acid composition of oxidized oil was determined as described earlier<sup>[15]</sup>.

**Animal treatment:** Male wistar albino rats were used in the study and obtained from animal care centre of King Saud University. After acclimatization the rats were randomly divided into six groups of six animals each.

- Group A: Basal diet
- Group B: Basal diet + cardamom (4%)
- Group C: Basal diet + cardamom (4%) + oxidized oil (4%)
- Group D: Basal diet + *N. sativa* (4%)
- Group E: Basal diet + *N. sativa* (4%) + oxidized oil (4%)
- Group F: Basal diet + oxidized oil

Animals were housed individually in a room with controlled temperature (22-23°C) and photoperiod (12 h/day). The average body weight of rat were 109.3±0.32 g when animals were started on experimental diet. They had free access to food and water. The basal diet consisted of (%) corn starch 62.07; sucrose 10.0; casein 14.0; fresh corn oil/oxidized corn oil 4.0; fiber 5.0; mineral mix 3.5; vitamin mix 1.0; DL methionine 0.18; choline barbiturate 0.25.

The spice diets were prepared by substituting 4% cardamom or *N. sativa* with corn starch.

**Blood and tissue sampling:** At the end of feeding period of 70 days, animals were fasted over night and sacrificed under light anaesthesia. The blood was drawn by cardiac puncture in heparinized vacutainer tubes. Small portion of the whole blood was used for the determination of RBC hemolysis. The remaining blood was centrifuged at 2000 rpm for 10 min to separate plasma. The organs were quickly dissected out into ice cold normal saline, dried, weighed and stored at -80°C. GSH and GST were estimated same day in 10% tissue homogenate.

**Red blood cell hemolysis:** *In vitro* red blood cell hemolysis was performed following the method of Buckingham<sup>[16]</sup>.

**Estimation of lipid peroxides:** Tissue peroxidation was determined by measuring Thiobarbituric Acid Reactive Substances (TBARS) formed, according to the method of Uchiyama and Mishara<sup>[17]</sup>. Briefly, 1 mL of 0.6% Thiobarbituric Acid (TBA) was added to 0.5 mL of tissue homogenate 10% w/v, containing 3 mL of 1% phosphoric acid. The mixture was kept in boiling water bath for 45 min. After cooling 4 mL *n*-butanol was added. The mixture was centrifuged at 4000 rpm and optical density of the supernatant was read at 535 and 520 nm. 1,1,3,3-Tetraethoxypropane was used as standard.

**Plasma enzymes:** The kinetic measurements of alanine aminotransferase and aspartate aminotransferase were carried out spectrophotometrically using commercially available diagnostic kits (Bio Mareux, France).

**Colourimetric determination of GSH and GST:** Reduced Glutathione (GSH) was determined by the method of Moron *et al.*<sup>[18]</sup> based on the reaction with 2,2-dithiobis (2-nitrobenzoic acid) to produce a compound with maximum absorption at 412 nm. Reduced glutathione in blood is easily oxidized to GSSG, so GSH was determined immediately after heparinized blood was withdrawn from study subjects. Glutathione-s-Transferase activity was measured with 1-chloro-2,4-dinitrobenzene<sup>[19]</sup>.

**Statistical analysis:** The data were analyzed by one way Analysis of Variance (ANOVA) followed by Tukey-Kramer Multiple Comparison Tests. Results were expressed as Mean±SD. The differences were considered significant at p<0.05.

## RESULTS

Analytical results of the fresh and oxidized corn oil is given in the Table 1. The changes in fatty acid composition were determined by GLC. Substantial fractions of linoleic and linolenic acids were lost during the heating process.

Table 1: Fatty acid composition and peroxide value (PV) of fresh and oxidized com oil

Fatty acid composition	Corn oil	
	Fresh	Oxidized
14:0	0.04	0.07
16:0	11.40	12.79
16:1	0.10	0.15
18:0	1.80	2.10
18:1	26.40	25.10
18:2	58.90	55.90
18:3	1.00	0.70
Others	-----	2.20
Peroxide value (meq kg <sup>-1</sup> )	2.25	389.80

Table 2: Final body weight, food intake, liver, kidney and heart weight of rats following consumption of diet

	Groups					
	A	B	C	D	E	F
Final body weight(g)*	369.47±36.21	345.48±27.21	335.52±40.27	345.18±33.07	340.20±34.16	305.25±29.88
Food intake (g/day)	22.03±02.16	22.16±02.88	23.34±03.71	22.13±03.40	21.74±02.14	19.99±01.43
Kidney weight (g)	1.86±00.37	1.69±00.17	1.69±00.28	1.90±00.19	1.79±00.21	1.58±00.22
Heart weight (g)	0.94±00.06	0.95±00.12	0.91±00.08	0.91±00.06	0.93±00.07	0.85±00.05
Liver weight (g)	9.09±00.83	9.67±01.17	9.54±01.00	9.67±01.10	9.01±00.25	8.96±00.95

Values are Mean±SD for 6 rats in each group, \* Initial body weight of all the rats were 109.3±0.32 (g). There were no significant differences in initial, final body weights, food intake, kidney, heart and liver weights among the six groups

Table 3: Percent hemolysis and plasma AST/ALT (IU/L) enzyme activity following consumption of diets

	Groups					
	A	B	C	D	E	F
% Hemolysis	5.93 ±1.18 <sup>a</sup>	4.97±0.68 <sup>ab</sup>	13.03±1.62 <sup>c</sup>	4.32±1.36 <sup>abd</sup>	9.83±1.96 <sup>c</sup>	22.98±2.97 <sup>f</sup>
AST	52.8±8.01 <sup>a</sup>	49.7±6.92 <sup>ab</sup>	56.4±7.84 <sup>a-c</sup>	48.1±4.79 <sup>a-d</sup>	54.7±6.25 <sup>a-e</sup>	79.4±8.07 <sup>f</sup>
ALT	28.0±3.98 <sup>a</sup>	26.6±1.75 <sup>ab</sup>	39.1±4.73 <sup>c</sup>	24.1±2.67 <sup>abd</sup>	37.8±4.01 <sup>ce</sup>	47.19±6.59 <sup>f</sup>

Table 4: TBARS concentration (n moles g<sup>-1</sup>) of tissues following consumption of diet

	Groups					
	A	B	C	D	E	F
Kidney	259.8±27.38 <sup>a</sup>	243.6±29.18 <sup>ab</sup>	274.2±23.71 <sup>a-c</sup>	231.2±16.66 <sup>abd</sup>	266.7±13.39 <sup>a-e</sup>	308.5±21.96 <sup>cf</sup>
Heart	204.0±16.49 <sup>a</sup>	198.7±17.57 <sup>ab</sup>	242.5±26.83 <sup>c</sup>	195.3±17.08 <sup>abd</sup>	235.3±23.49 <sup>a-e</sup>	254.8±24.41 <sup>cf</sup>
Liver	312.8±20.08 <sup>a</sup>	311.0±17.94 <sup>ab</sup>	337.2±15.81 <sup>a-c</sup>	301.8±15.13 <sup>abd</sup>	326.3±24.88 <sup>a-e</sup>	388.7±15.74 <sup>f</sup>

Table 5: Tissue Glutathione (GSH, μ mole g<sup>-1</sup> tissue) following consumption of diets

	Groups					
	A	B	C	D	E	F
Heart	1.54±0.37 <sup>a</sup>	1.59±0.41 <sup>ab</sup>	1.24±0.21 <sup>a-c</sup>	1.86±0.40 <sup>abd</sup>	1.34±0.21 <sup>a-e</sup>	1.04±0.21 <sup>abcef</sup>
Kidney	3.75±0.54 <sup>a</sup>	3.97±1.00 <sup>ab</sup>	3.00±0.20 <sup>a-c</sup>	4.08±1.01 <sup>a-d</sup>	3.39±0.72 <sup>a-e</sup>	2.53±1.19 <sup>acef</sup>
Liver	5.04±1.09 <sup>a</sup>	5.52±0.63 <sup>ab</sup>	4.02±0.49 <sup>a-c</sup>	5.62±0.68 <sup>abd</sup>	4.89±0.74 <sup>a-e</sup>	3.08±0.84 <sup>cf</sup>

Table 6: Tissue Glutathione-s-transferase (GST, μ mole min<sup>-1</sup> g<sup>-1</sup> tissue) following consumption of diets

	Groups					
	A	B	C	D	E	F
Kidney	6.31±0.55 <sup>a</sup>	7.02±1.09 <sup>ab</sup>	5.8±0.52 <sup>a-c</sup>	7.39±1.50 <sup>a-d</sup>	5.39±0.99 <sup>a-e</sup>	3.96±0.23 <sup>f</sup>
Liver	17.69±2.10 <sup>a</sup>	18.18±2.30 <sup>ab</sup>	16.26±1.00 <sup>a-c</sup>	19.1±0.98 <sup>a-d</sup>	16.04±1.99 <sup>a-e</sup>	14.63±1.60 <sup>acef</sup>

Values are Mean±SD for 6 rats in each group. Within the same row values not sharing the same superscript are significantly different (p<0.05)

Food intake, body weight, heart, kidney and liver weights were not affected significantly by feeding oxidized oil to the rats (Table 2).

Oxidized oil fed rats had increased rate of RBC hemolysis than Groups A-E (Table 3). However, the addition of cardamom/*N. sativa* to the oxidized oil diet caused significant reduction in the rate of hemolysis. The decreased plasma AST/ALT activity (Table 3) was also found to be lowered in Groups C and E compared to oxidized oil fed rats.

Similarly, the addition of oxidized oil to the diet resulted in significant elevation of liver, kidney and heart TBARS as compared to Groups A-E (Table 4). *N. sativa* fed rats had lowest level of TBARS in liver, heart and kidney. Addition of oxidized oil to the cardamom supplemented group showed an increase in TBARS of heart compared to Group A, B and D, while kidney and liver showed an increased level of TBARS only with *N. sativa* fed rats.

Addition of *N. sativa* (Group D) to the basal diet caused a significant increase in GSH of heart, kidney and liver compared to oxidized oil (Group F) fed rats (Table 5). Heart and liver GSH of Group D also showed significant increase compared to Group C. Somewhat similar changes were noticed in the liver and kidney of cardamom fed rats. Feeding rats with diet containing oxidized oil and supplemented with *N. sativa* showed significant increase only in liver compared to Group F. Liver GST is significantly high in Groups B and D compared to Group F (Table 6). Supplementation of *N. sativa* or cardamom to the basal diet causes a significant increase in kidney GST when compared either to Group C, E and F.

## DISCUSSION

Oxidative stress is implicated in the pathology of many diseases and aging. The susceptibility of humans

and animals to oxidative damage is dependent on the balance between oxidative stress and antioxidant defense capacity. Thermally oxidized fats contain a large number of lipid oxidation products that are known to effect animal metabolism in many ways<sup>[2]</sup>. Highly oxidized fats usually contain significantly less polyunsaturated fatty acids and tocopherols than the equivalent fresh oils<sup>[20,21]</sup>.

The quality of the corn oil declined considerably through oxidation process, as there was an apparent decrease in polyenoic acid. The peroxide value, an index of lipid peroxidation was typically higher in oxidized corn oil than fresh corn oil.

In several studies rats fed oxidized oils showed growth reduction due to oxidative stress and reduced digestibility of nutrients<sup>[22-25]</sup>. In present study rats grow well and appeared healthy on oxidized oil supplemented diets. No marked differences in food intake, body weight, heart, kidney and liver weights were evident at the end of feeding regime. The pattern observed in our study is in close agreement with the finding of Huang *et al.*<sup>[26]</sup> and Liu and Huang<sup>[27]</sup>. Present results thus indicates that the fat used is moderately oxidized compared with those used in other studies. Yoshida and Kajimoto<sup>[22]</sup> observed a marked decrease in growth of rats fed 15% oxidized oil (pv 358.9). This may be due to the high percentage of oxidized oil used in their study.

It has been reported earlier that the ingestion of Oxidized Frying Oil (OFO) causes significant elevation of RBC *in vitro* hemolysis<sup>[26]</sup>. Hemolysis is one of the common symptoms of vitamin E deficiency. Liu and Huang<sup>[27]</sup> observed increased consumption and metabolism of  $\alpha$ -tocopherol by intake of diet containing OFO. Present results showed a significant reduction in the rate of hemolysis after addition of cardamom/ *N. sativa* to the oxidized oil supplemented diets. This indicates an active antioxidant role of thymoquinone and eugenol present in *N. sativa* and cardamom, respectively.

Damage due to the toxicity of dietary lipids, lipid hydroperoxides on liver and other organs has been observed by several investigators<sup>[28,29]</sup>. Liver damage can be assessed by leakage of enzyme such as ALT and AST and lactate dehydrogenase<sup>[30,31]</sup>. It is well documented that OFO fed animals had significantly higher activities of ALT and AST compared to control<sup>[32]</sup>. We observed a significant decrease in ALT and AST activities in rats fed diet containing oxidized oil supplemented with cardamom/*N. sativa*.

Present results are well in agreement with the reports of Huang *et al.*<sup>[26]</sup>, Liu and Huang<sup>[27]</sup>, Andrews *et al.*<sup>[33]</sup> and Iritani *et al.*<sup>[34]</sup> who showed that the ingestion of oxidized oil results in higher tissue lipid peroxidation as judged by higher tissue TBARS level. Many spices and herbs are strong scavengers of active oxygen species<sup>[3]</sup>. Eugenol, a compound present in cardamom and many other

spices<sup>[14]</sup> are known as one of the strong radical scavengers due to its phenolic hydroxyl group<sup>[3]</sup>. Thymoquinone, the main constituent of the volatile oil from *N. sativa* seed is reported to inhibit eicosanoid generation in leukocyte, non-enzymatic peroxidation in ox brain phospholipid liposomes and membrane lipid peroxidation<sup>[35]</sup>. It has also been demonstrated by Pulla Reddy and Lokesh<sup>[7]</sup> that the spice principles such as curcumin from turmeric and eugenol from cloves can effectively inhibit lipid peroxidation in rat liver by enhancing the antioxidant enzyme activities.

Glutathione, a substrate for glutathione peroxidase, helps to prevent radical initiation and thus acts as an important defense mechanism in the living cells. As water soluble antioxidant GSH also helps to maintain antioxidant status of the body by keeping the ascorbate and  $\alpha$ -tocopherol in their reduced states<sup>[36,37]</sup>. Glutathione-s-transferase also plays a potentially important role in liver by eliminating some toxic compounds by conjugating them with glutathione. GSH and GST levels were restored in rats fed on oxidized oil diets supplemented with cardamom or *N. sativa*. This finding points out to the fact that *N. sativa* and cardamom do have some antioxidant property, that enables the rat to protect themselves from the deleterious effects of oxidized oil.

## CONCLUSIONS

Present results showed that both *N. sativa* and cardamom possesses antioxidant properties. However the antioxidant property of *N. sativa* is definitely more pronounced than cardamom. This could be due to additional antioxidant property of C20:2, unsaturated fatty acid found in *N. sativa* as reported by Houghton *et al.*<sup>[37]</sup>. Further studies are needed to explore the possibilities as why *N. sativa* has greater antioxidant effect than cardamom.

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