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# *IN VITRO* STUDY ON THE ANTIOXIDANT ACTIVITY OF SOME MEDITERRANEAN CULINARY SPICES AND THEIR LYSOSOMAL MEMBRANE STABILITY

**Mahgoub M. Ahmed[1] and Mohamed B. Abou-Donia[2]**

[1]*Molecular Drug Evaluation, National Organization for Drug Control and Research, Giza, Egypt*
[2]*Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, USA*

## ABSTRACT

The *in vitro* scavenging activities of four Mediterranean spices: allspice, sage, anise, and caraway on $H_2O_2$ and DPPH$^{\bullet}$ radicals, microsomal lipid peroxidation, and the iron-chelating ability were studied. In addition, the protective effect of the four spices against cisplatin-induced lysosomal membrane damage was evaluated. All these spices scavenged $H_2O_2$ and DPPH$^{\bullet}$ radicals and inhibited the lipid peroxidation process. The iron ions were chelated by these spices. In addition, the four spices at two concentrations (1 and 5 mg/ml) protected the lysosomal membrane against cisplatin by decreasing the release of acid phosphatase (ACP) and N-acetyl-β-D-glucosaminidase (NAG). The antioxidant activity of these spices may be due to radical-scavenging and metal-chelating activity. These results concluded that these spices can be used as a source of natural antioxidants and dietary supplements to prevent free radical diseases.

**Keywords**: allspice, sage, anise, caraway, antioxidant activity, cisplatin, lysosomes, ACP, NAG.

## INTRODUCTION

Reactive oxygen species (ROS) are generated by many redox processes that normally occur in metabolism of aerobic cells. If not eliminated, ROS can attack important biological molecules, such as lipids, proteins, DNA, enzymes, and RNA (Jung et al., 1999 and Yu et al, 2002). Thus, ROS are involved in a number of degenerative diseases such as cancer, cirrhosis, diabetes, and Alzheimer's (Arouma, 1998; Baynes, 1991; Buyukbalci & Nehir EI, 2008).

ROS contribute to cell inflammatory changes and to the nephrotoxicity induced by xenobiotics (Virgili et al., 1998; Ahmed & Safaa, 2008). Cisplatin induces oxidative stress (Ahmed, 2007), lipid peroxidation (Matsushima et al., 1998; Ahmed, 2007), and release of lysosomal enzymes (Cathepsin D, DNase II, and acid phosphatase) from renal lysosomes (Ahmed, 2007).

Recently, it has been proposed that the lysosomes in renal tubular cells may play a role in the nephrotoxicity via the release of hydrolytic lysosomal enzymes into the cytosol. Agents which promote the release of lysosomal enzymes *in vitro* and/or *in vivo* are known as labilizers such as $CCl_4$ (Abdel-Samad et al., 2006; Abdel-Meseih et al., 2006), cisplatin (Ahmed, 2007), detergents, various free radical generators such as UV and X-ray, and oxidizing agents (De Duve et al, 1962).

Spices are common food adjuncts that impact flavor, aroma, or piquancy to foods. Spices are consumed in a variety of combinations depending on taste preferences. Allspice, *Pimenta dioica* (L.) Merrill (Myrtaceae) is known as the spice with three flavors (Nel, 1994; Leung, 1980; Al-Rehaily et al., 2002). Allspice is used as a spice and condiment it is also used in the treatment of digestive ailments, abdominal pain, obesity, hyperglycemia, and high blood pressure in traditional medicine (Asprey & Thornton, 1953). Allspice fruit contains flavonoids, tannins, sterols, and/or triterpene and volatile oil (Al-Rehaily et al., 2002). Also, new compounds from allspice together with 11 polyphenols were separated (Miyajima et al. 2004). Two new compounds exhibited strong DPPH radical-scavenging activity and inhibitory effects on lipid peroxidation of liposomes.

Anise, *Pimpinella anisum* (L.) (Umbelliferae) is an annual herb growing in Egypt and many other warm regions of the world (Pourgholami et al., 1999; Zargari, 1989). It was reported that anise had several effects on digestive, neurological, and respiratory disorders (Aboabrahim, 1970).

Sage, *Saliva officinalis* (L.) (Lamiaceae) is a common and widely used aromatic and medicinal plant native to Mediterranean countries. Sage has a variety of bioactivities such as anti-inflammatory, antioxidant, hypoglycemic, and anti-mutagenic activities (Cuvelier et al., 1994; Wang et al., 1996; Hollmann et al., 1999; Baricevic & Bartol, 2000; Zupko et al., 2001; Alarcon-Aguilar et al., 1998; Lima et al., 2005).

Caraway, *Carum carvi* (L.) (Umbelliferae) has been used as a popular aromatic herb and spice (Norman, 1990; Wichti, 1994), its fruits has been used for medicine and in cooking (Wichti, 1994). For medicinal purposes, it is used for relieve flatulent indigestion, colic, and bronchitis (Norman, 1990; Wichti, 1994).

The objective of this study was to evaluate the antioxidant activity of four commonly used culinary spices: allspice, sage, anise, and caraway, as well as the protective effect of allspice on the lysosomal enzymes in cisplatin-induced renal damage *in vitro*.

## MATERIALS AND METHODS

### 1.Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), hydrogen peroxide, thiobarbituric acid (TBA), *tert*-butylhydroxyanisole (BHA), ρ-nitrophenyl phosphate, and ρ-nitrophenyl-N-acetyl-β-D-glucosaminide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cisplatin (1mg/ml) Onco-Tain DBL was from MaynePharm PLC. UK.

### 2. Animals

Male Spargue-Dawley rats, weighing 200-230 g, were obtained from Zivic-Miller Laboratories (Allison Park, PA) and housed in Duke University Medical Center vivarium on 12 hr dark-light cycle. The rats were allowed food and water *ad libitum*. They were provided with a nutritionally adequate standard laboratory diet.

### 3. Animals' diet

The basal diet consists of casein 10%, cotton seed oil 4%, salt mixture 4%, vitamin mixture 1%, carbohydrates (sucrose, starch 1:1) 80.8%, and choline chloride 0.2% (American Institute of Nutrition, 1980).

### 4. Sample preparations

Four commonly used Egyptian culinary dried spices including: allspice, anise, caraway, and sage were purchased from a local market in Cairo, Egypt. The dried spices were ground and then extracted with 90% methanol according to the procedures described by Halvorsen et al. (2002) and Tasi et al. (2007). The resulting supernatant was then lyophilized in a freeze dryer (Snijders Scientific, model W5fm). The

lyophilized extracts were kept in brown sealed vials that were kept in a freezer until tested.

**5. DPPH$^{\bullet}$ scavenging assay**

The antioxidant activities of the extracts were estimated by Chen et al. (1999) method with a slight modification by Marwah et al. (2007). For a typical reaction, 2 ml of 100 μM DPPH$^{\bullet}$ solution in ethanol were mixed with 2 ml of 50, 100, and 200 μg/ml of extract. The reaction mixture was incubated in the dark for 15 min., and, thereafter the optical density was recorded at 517 nm against the blank. For the control, 2 ml of DPPH$^{\bullet}$ solution in ethanol was mixed with 2 ml of ethanol, and the optical density of the solution was recorded after 15 min. The decrease in optical density of DPPH$^{\bullet}$ on addition of test samples in relation to the control was used to calculate the antioxidant activity. The assay was carried out in triplicate and the results were presented as means$\pm$SD. The percent inhibition activity was calculated from: %I = [1-(absorbance of sample / absorbance of control)] x 100.

**6. Hydrogen peroxide assay**

Hydrogen peroxide scavenging activity was measured by the method of Wettasinghe & Shahidi (2000). 1 ml of sample at different concentrations was mixed with 2.4 ml of 0.1 M phosphate buffer (pH 7.4) and then 0.6 ml of a 43 mM solution of $H_2O_2$ in the sample buffer was added. After 40 min. the absorbance values at 230 nm of the reaction mixture were recorded against a blank solution containing phosphate buffer without $H_2O_2$ for each sample. For each concentration, a separate blank sample was used for background substraction. The percentage inhibition activity was calculated from: % I= $[(A_0-A_1)/A_1]$ x 100, where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the extract/standard. All tests were done in triplicate and the results were presented as means $\pm$ SD.

**7. Metal chelating activity**

The chelating of ferrous ions by the spice methanol extracts and standard was estimated by the method of Dinis et al. (1994) and Gulcin et al. (2003). Briefly, extracts (50, 100, and 200 μg/ml) were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), and the mixture

was shaken vigorously and left at room temperature for 10 min. the absorbance of the solution was then measured using a spectrophotometer at 562 nm. All samples were run in triplicate. The percentage of inhibition of ferrozine-$Fe^{2+}$ complex formation was calculated using the following formula: $\%I = [(A_0-A_1)/A_0] \times 100$, where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the presence of the samples or standards. The control does not contain $FeCl_2$ and ferrozine, complex formation molecules.

## 8. Microsomal lipid peroxidation

### 8.1. Preparation of microsomes

Rats were killed by cervical decapitation, livers were removed and the liver microsomes were prepared according to the method of Cai et al. (2003). Microsomal protein content was determined by the method of Lowry et al. (1951).

### 8.2. $Fe^{2+}$/ascorbate model system

The reaction mixture contained 1 mg microsomal protein/ml, 1.6 mM ascorbic acid, and the four spices or phosphate buffer (for control reactions). The reaction was started with the addition of 0.2 mM $FeSO_4$, followed by incubation at 37°C for 30 min. Lipid peroxidation products were measured according to the method of Buege & Aust (1978). 1 ml of phosphoric acid at 1% and 300 µl of 0.6% thiobarbituric acid (W/V) were added and the mixture was heated in a water bath at 100°C for 45 min. The absorbance samples were measured at 535 nm. The inhibitory effect of samples was calculated according to the following equation: $\%I = [(A_0-A_1)/A_1] \times 100$, where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the extract/standard. All tests were done in triplicate and the results were presented as means $\pm$ SD.

## 9. Lysosomal membrane stability

### 9.1. Preparation of rat kidney lysosomes

Renal cortical lysosomes were prepared by the method described by Shibko & Tappel (1965), Kojima et al. (1987), and Win-Aung et al. (1998). The lysosomal fractions were resuspended in sucrose/EDTA to obtain 20 mg/ml concentration. Protein content was measured by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

**9.2. Incubation of the lysosomes with the samples**

The lysosomal fractions were incubated with cisplatin (0.2 mg/ml) or with cisplatin (0.2 mg/ml) and each spice by two concentrations; 1 and 5 mg/ml. The reaction mixture was incubated for 30 min. at 37°C. After incubation, the lysosomal fractions were quickly cooled in an ice bath, and centrifuged at 15,000 x g for 10 min. The supernatant was used for measuring N-acetyl-β-D-glucosaminidase (NAG) and acid phosphatase (ACP) activities.

**9.3. Determination of Enzyme activity**

NAG activity was assayed by the method of Hasebe (1968) using ρ-nitrophenyl-N-acetyl-β-D-glucosaminide as a substrate. ACP activity was determined by the method of Igarashi & Hollander (1968) using ρ-nitrophenyl phosphate as substrate. One unit of activity of NAG and ACP was defined as the amount of enzyme that liberated 1 nmol of ρ-nitrophenyl/ml/mg protein and 0.06 mmol of ρ-nitrophenyl/ml/mg protein at 37°C, respectively.

## RESULTS AND DISCUSSION

**1. Free radical-scavenging activity**

The data obtained are presented in Table 1. All the test spices dose-dependently scavenged $DPPH^{\bullet}$ free radical. Among four spices under investigation, allspice had the highest hydrogen-donating capacity against the $DPPH^{\bullet}$ radical. Ranking of the spices with respect to their radical scavenging activity against the $DPPH^{\bullet}$ radical was in the order allspice> sage> anise> caraway.

The radical-scavenging activity of antioxidants may be influenced by the radical system and other testing conditions. Two or more radical systems are needed to better study a selected antioxidant for its radical-scavenging properties. $DPPH^{\bullet}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997; Gulcin et al., 2003). The reduction capacity of $DPPH^{\bullet}$ radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, $DPPH^{\bullet}$ is often used as a substrate to evaluate antioxidative activity of antioxidants (Duh et al., 1999).

**Table 1: Free radical scavenging activity of water extract of allspice, sage, anise, caraway, α-tocopherol, and *tert*-butylhydroxyanisole.**

| Plant Extract | Concentration | %I |
|---|---|---|
| **Allspice** | | |
| | 50 µg/ml | 68.59 ± 1.15 |
| | 100 µg/ml | 77.52 ±1.16 |
| | 200 µg/ml | 92.45 ±0.28 |
| **Sage** | | |
| | 50 µg/ml | 27.95 ± 1.51 |
| | 100 µg/ml | 71.82 ± 0.83 |
| | 200 µg/ml | 78.68 ± 2.10 |
| **Anise** | | |
| | 50 µg/ml | 26.56 ± 1.22 |
| | 100 µg/ml | 53.97 ± 0.35 |
| | 200 µg/ml | 62.28 ± 0.96 |
| **Caraway** | | |
| | 50 µg/ml | 7.16 ± 1.83 |
| | 100 µg/ml | 14.63 ± 0.74 |
| | 200 µg/ml | 54.27 ± 1.67 |
| **α-Tocopherol** | | |
| | 50 µg/ml | 96.92 ± 1.19 |
| | 100 µg/ml | 99.69 ± 0.13 |
| | 200 µg/ml | 100.00 ± 0.00 |
| ***tert*-Butylhydroxyanisole** | | |
| | 50 µg/ml | 52.96 ± 1.49 |
| | 100 µg/ml | 63.51 ± 1.22 |
| | 200 µg/ml | 75.91 ± 0.93 |

Each value is expressed as the mean ± standard deviation of three replicate analyses

## 2. $H_2O_2$ scavenging activity

Table 2 shows the scavenging activity of the sample on $H_2O_2$. Samples were capable of scavenging $H_2O_2$ in concentration-dependent fashion. The concentrations of the samples could not completely remove $H_2O_2$ from the medium. The highest inhibition value (77.2 ± 2.7) was obtained for allspice at 200 µg/ml concentration. Samples at a concentration of 200 µg/ml were ranked according to their $H_2O_2$ scavenging capacity in the following order: allspice>sage>anise>caraway. α-Tocopherol was more effective than samples at the same concentrations.

Yen and Duh (1994) reported that $H_2O_2$ has only a weak activity to initiate lipid peroxidation, but the activity as an active oxygen species comes from its potential to produce the highly reactive hyrdroxyl radical through the Fenton reaction.

Since phenolic compounds present in these spices are good electron donors, they may accelerate the conversion of $H_2O_2$ to $H_2O$ (Kumaran & Karunakaran, 2007).

**Table 2: Hydrogen peroxide scavenging activities of water extracts of allspice, sage, anise, caraway, α-tocopherol, and *tert*-butylhydroxyanisole.**

| Plant extract | Concentration | %I |
|---|---|---|
| **Allspice** | | |
| | 50 µg/ml | 35.44 ± 2.74 |
| | 100 µg/ml | 53.42 ±1.16 |
| | 200 µg/ml | 77.22 ± 0.76 |
| **Sage** | | |
| | 50 µg/ml | 33.29 ± 1.22 |
| | 100 µg/ml | 51.52 ± 0.58 |
| | 200 µg/ml | 71.14 ± 1.32 |
| **Anise** | | |
| | 50 µg/ml | 31.27 ± 1.66 |
| | 100 µg/ml | 52.41 ± 2.32 |
| | 200 µg/ml | 70.51 ± 1.58 |
| **Caraway** | | |
| | 50 µg/ml | 27.59 ± 0.88 |
| | 100 µg/ml | 47.34 ± 1.16 |
| | 200 µg/ml | 60.63 ± 1.44 |
| **α-Tocopherol** | | |
| | 50 µg/ml | 53.29 ± 1.00 |
| | 100 µg/ml | 60.76 ± 1.58 |
| | 200 µg/ml | 79.37 ± 1.87 |
| ***tert*-Butylhydroxyanisole** | | |
| | 50 µg/ml | 39.37 ± 2.85 |
| | 100 µg/ml | 52.41 ± 1.91 |
| | 200 µg/ml | 61.39 ± 1.44 |

Each value is expressed as the mean ± standard deviation of three replicate analyses

## 3. Metal chelating activity

Table 3 presents that all samples have chelating effect against ferrous ions and this effect is dose-dependent. Sage has the highest chelating activity (76.2 ± 1.4) at the concentration of 200 µg/ml. Chelating activity of the four samples at the concentration of 200 µg/ml were recorded and found to be in the following order: sage>allspice>anise>caraway. α-tocopherol and BHA exerted more chelating activity than the samples at 200 µg/ml on $Fe^{2+}$. These results suggest the potential activity of allspice, sage, anise, and caraway to prevent oxidative damage from free radical mediated oxidation.

Iron is essential for life because it is required for oxygen transport, respiration, and the activity of many enzymes (Duh et al., 1999; Karakaya et al., 2004). However, metals are well-known initiators of unwanted oxidative reactions in lipids, proteins, and other

cellular components (Karakaya et al., 2004). In addition, iron is capable of generating free radicals from peroxides by Fenton reactions, and minimization of the $Fe^{2+}$ concentration in the Fenton reaction affords protection against oxidative damage (Lai et al., 2001). Chelating agents may reduce the availability of transition metals and inhibit the radical-mediated oxygen chain reactions in biological or food systems and consequently improve human health and food quality, stability, and safety. Ferrozine can quantitatively form a complex with $Fe^{2+}$. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red color of the complex. Measurement of color therefore allows estimating the metal chelating activity of the coexisting chelator (Yamaguchi et al., 2000; Gulcin et al., 2003).

**Table 3: Metal chelating effect of water extracts of allspice, sage, anise, caraway, α-tocopherol, and *tert*-butylhydroxyanisole.**

| Plant extract | Concentration | %I |
|---|---|---|
| **Allspice** | | |
| | 50 µg/ml | 18.81 ± 1.20 |
| | 100 µg/ml | 54.99 ± 0.65 |
| | 200 µg/ml | 72.94 ± 0.54 |
| **Sage** | | |
| | 50 µg/ml | 22.90 ± 1.12 |
| | 100 µg/ml | 54.99 ± 1.76 |
| | 200 µg/ml | 76.24 ± 1.43 |
| **Anise** | | |
| | 50 µg/ml | 35.61 ± 0.57 |
| | 100 µg/ml | 56.57 ± 1.30 |
| | 200 µg/ml | 68.49 ± 1.19 |
| **Caraway** | | |
| | 50 µg/ml | 35.61 ± 0.22 |
| | 100 µg/ml | 60.95 ± 0.90 |
| | 200 µg/ml | 63.03 ± 0.87 |
| **α-Tocopherol** | | |
| | 50 µg/ml | 55.56 ± 0.97 |
| | 100 µg/ml | 61.95 ± 0.97 |
| | 200 µg/ml | 79.04 ± 1.08 |
| ***tert*-Butylhydroxyanisole** | | |
| | 50 µg/ml | 64.54 ± 1.19 |
| | 100 µg/ml | 79.54 ± 0.57 |
| | 200 µg/ml | 86.07 ± 1.38 |

Each value is expressed as the mean ± standard deviation of three replicate analyses

## 4. Inhibitory effect of samples on lipid peroxidation

As shown in Table 4, all samples dose-dependently inhibited lipid peroxidation induced by $FeCl_2$ in the rat kidney. At a concentration of 2 mg/ml, all samples showed more than 50% inhibitory effect on lipid peroxidation. Sage at concentration of 2 mg/ml has the highest inhibitory effect (79.57 ± 0.96) against lipid peroxidation. The inhibitory effect of samples were recorded and found to be in the following order: sage>allspice>anise>caraway.

The antioxidant mechanism of these spices may involve trapping the initiating peroxy radical and/or hydroxy radicals. The first event of lipid peroxidation is oxygen absorption and a final product of the peroxidation is TBARS (Buge & Aust, 1978). In the present work, TBARS method was used to evaluate the antioxidative activity of the four spices *in vitro*. The $Fe^{2+}$/ascorbate model system was used to initiate hydroxyl radicals by Fenton reaction (Halliwell & Gutteridge, 1987), which initiates the microsomal lipid peroxidation.

**Table 4.Inhibitory effect of water extract of four spices against microsomal lipid peroxidation induced by $Fe^{2+}$/ascorbate system.**

| Plant Extract | Concentration | %I |
|---|---|---|
| **Allspice** | | |
| | 1.0 mg/ml | 31.84 ± 0.96 |
| | 1.5 mg/ml | 63.11 ± 0.96 |
| | 2.0 mg/ml | 76.20 ± 0.80 |
| **Sage** | | |
| | 1.0 mg/ml | 33.82 ± 1.72 |
| | 1.5 mg/ml | 59.09 ± 1.44 |
| | 2.0 mg/ml | 79.58 ± 0.96 |
| **Anise** | | |
| | 1.0 mg/ml | 21.76 ± 1.44 |
| | 1.5 mg/ml | 50.54 ± 1.23 |
| | 2.0 mg/ml | 61.90 ± 1.45 |
| **Caraway** | | |
| | 1.0 mg/ml | 18.19 ± 1.05 |
| | 1.5 mg/ml | 45.25 ± 0.96 |
| | 2.0 mg/ml | 58.77 ± 0.96 |
| **α-Tocopherol** | | |
| | 1.0 mg/ml | 68.09 ± 0.96 |
| | 1.5 mg/ml | 76.71 ± 0.72 |
| | 2.0 mg/ml | 83.15 ± 1.16 |
| ***tert*-Butylhydroxyanisole** | | |
| | 1.0 mg/ml | 57.82 ± 1.73 |
| | 1.5 mg/ml | 66.88 ± 0.57 |
| | 2.0 mg/ml | 74.92 ± 0.83 |

Each value is expressed as the mean ± standard deviation of three replicate analyses

Phenolics are very important plant constituents because of their radical-scavenging ability due to their hydroxyl groups. Anise water extract has 30 μg gallic acid equivalents of phenols (Gulcin et al., 2003). In addition, total phenolics content in the cold-pressed black caraway seed oil was 3.53 mg gallic acid/g oil (Yu et al., 2005). Total phenolics content in allspice was 1.79 mg gallic acid equivalent/ml extract (Tasi et al., 2007). Total phenolics in sage infusion were 330 mg catechin equivalent per liter of herbal infusion (Buyukbalci & Nehir, 2008). The antioxdiant properties of the four spices under investigation may arise from their radical-scavenging and metal chelating activities. Most antioxidant capacities of these spices are attributable to the total phenolics content contained in these spices.

## 5. Effect of allspice on cisplatin-induced renal damage

As shown in Table 5, the release of ACP and NAG from the incubation of lysosomes with cisplatin (0.2 mg/ml) alone is increased by 95.5% and 134.8.9%, respectively, as compared to the control. Various data indicate that cisplatin induces oxidative stress, lipid peroxidation, and DNA damage (Matsushima et al., 1998; Ahmed, 2007).

**Table 5.Effect of of water extract of four spices against cisplatin (CIS)-induced release of acid phosphatase (ACP) & N-B- acetyl glucosaminidase (NAG) from rat kidney lysosomes.**

| | ACP (nmol/ml/mg protein) | NAG (nmol/ml/mg protein) |
|---|---|---|
| **Control** | 34.65±8.5 | 9.45±2.3 |
| **CIS** | 70.67±8.6a | 25.74±.3a |
| **CIS+Allspice(1mg)** | 43.76±6.8b | 12.65±3.1b |
| **CIS+Allspice(5mg)** | 31.39±9.7b | 10.12±2.4b |
| **CIS+Sage(1mg)** | 38.29±6.7b | 9.80±4.3b |
| **CIS+Sage(5mg)** | 35.61±7.6b | 9.20±4.1b |
| **CIS+Anise(1mg)** | 40.64±7.7b | 12.99±3.5b |
| **CIS+Anise(5mg)** | 36.74±6.1b | 10.11±3.8b |
| **CIS+Caraway(1mg)** | 45.22±5.8b | 13.85±4.2b |
| **CIS+Caraway(5mg)** | 34.94±8.4b | 10.00±5.7b |

The values are expressed as means ± SD of triplicate tests [a] $p<0.05$, (Student´s *t test*) significantly different from control group. [b] $p<0.05$, significantly different from CIS group. CIS: cisplatin

On the other hand, upon addition of the spices by two concentrations (1 and 5 mg/ml) with the cisplatin, the release of ACP and NAG from lysosomes was suppressed. This inhibition effect of the spices on cisplatin was dose-dependent. These effects may contribute to the antioxidant activity of the spices, especially against lipid peroxidation which induces the oxidation of unsaturated fatty acids in biological membranes. The oxidation of unsaturated fatty acid in biological membranes by free radicals leads to a decrease in membrane fluidity and disruption of membrane structure and function (Haragushi et al., 1997). Also, Ahmed (2007) has reported that the release of ACP, DNase II, RNase II, and Cathepsin D was increased after the treatment with cisplatin (5 mg/kg, ip) *in vivo*.

**Conclusion**

The four spices: allspice, sage, anise, and caraway have strong antioxidant activities including DPPH radical and hydrogen peroxide scavenging, metal chelating activity, and inhibition of lipid peroxidation of microsomes as compared with different standards such as BHA and α-tocopherol. In addition, the four spices by two concentrations; 1 and 5 mg/ml appeared to be protected the lysosomal membrane against cisplatin-induced renal damage. The results of this study show that the methanol extracts of these spices can be used as sources of natural antioxidants, as food supplements, or can be taken with cisplatin to reduce its nephrotoxicity. Further research is needed to investigate the potential biological activities of these spices.

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## دراسات معملية على النشاط المضاد للاكسدة و المثبط لاغشية الليسوسوم لبعض توابل حوض البحر المتوسط

**[1]محجوب محمد احمد و[2] محمد باهى ابو دنيا عطية***
[1]شعبة التقييم الدوائى الجزيئى-الهيئة القومية للرقابة و البحوث الدوائية-مصر
[2]قسم الفارماكولوجى و بيولوجيا السرطان-جامعة ديوك-امريكا

تم تقييم النشاط الكاسح معمليا للشقوق الحرة فوق اكسيد الهيدروجين و الداى فينايل بكرايل هيدرازيل و تثبيط اكسدة ليبيدات الميكروسوم لاربعة توابل منتشرة فى حوض البحر المتوسط (الكبابة الصينى و المريمية و الينسون و الكراوية) و كذلك قدرة هذة التوابل على ربط الحديد. بالاضافة الى ذلك فقد تم دراسة التاثير الواقى لهذة التوابل ضد مركب السيسبلاتين و الذى يسبب تلف لاغشية الليسوسوم. كل هذة التوابل استطاعت كسح الشقوق الحرة فوق اكسيد الهيدروجين و الداى فينايل بكرايل هيدرازيل و تثبيط اكسدة ليبيدات الميكروسوم. كذلك ايونات الحديد تم ربطها بهذة التوابل.

بالاضافة الى ذلك فإن هذة التوابل استطاعت حماية اغشية الليسوسوم من التاثير الضار للسيسبلاتين و ذلك بتقليل معدل انسياب انزيمى الفوسفاتيز الحامضى و ن-اسيتيل-د-جلوكوزامينيديز. النشاط المضاد للاكسدة لهذة التوابل ربما يكون راجع من نشاطها الكاسح للشقوق و ربطها للحديد. هذة النتائج توضح ان هذة التوابل يمكن استخدامها كمصدر للمواد المضادة للاكسدة و كمصدر غذائى للوقاية من الامراض التى تسببها الشقوق الحرة.