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A COMPARATIVE STUDY OF CUMIN ALDEHYDE AND PARA-CYMENE EFFECTS ON MICE LIVER TISSUE'S ANTIOXIDANT ACTIVITY AFTER 24 HOURS OF INCUBATION *INVITRO*

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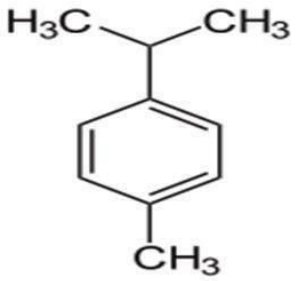
ABSTRACT

The medicinal plants active components have valuable biochemical effects. The aim of this study was to assess the effects of cumin aldehyde and para-cymene, the two standard active ingredient of cumin, on the activity of antioxidant enzymes and the levels of oxidative damage biomarkers in the rat liver. Enzyme activities and malondialdehyde levels was evaluated by spectrophotometry and HPLC methods respectively. The results showed that both compounds in the intermediate concentration range (40 and 80 µM) have the ability to increase the liver tissue antioxidant activity Such that the superoxide dismutase, catalase and glutathione peroxidase activities shows significant increasing 34%, 37% and 48% respectively under cumin aldehyde effect compared to the control condition. The activity of these enzymes under the para-cymene influence showed a significant increase of 65%, 17% and 41% compared to the control condition. On the other hand, the levels of oxidative damage biomarkers decreased significantly in these concentrations which could be due to the positive effect of antioxidant activity in inhibiting the macromolecular oxidative damages. Therefore, since these compounds have significant stimulatory antioxidant properties on the liver, they can be used to protect liver against oxidative stress and disease.

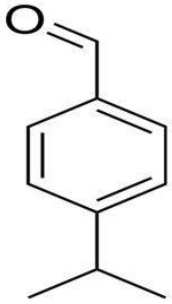
KEYWORDS: Cumin Aldehyde, Para-Cymene, Antioxidant Enzymes, Biomarkers of Oxidative Damage

Cumin (*Cuminum cyminum*) is a plant in the **family** Apiaceae and a member of the parsley family, native from East Mediterranean to the India. The cumin plant grows to 30–50 cm tall and is harvested by hand (Allahghadri,2010). Its essential oil contains high levels of phenolic compounds, mainly cumin aldehyde, and para-cymene (Zargari, 1360).

Cumin aldehyde (4 - isopropyl benzaldehyde) (Fig. 1 a) is an organic compound with the molecular formula $C_{10}H_{12}O$. Cumin aldehyde is a benzaldehyde with an isopropyl group at its 4 position and is the main component of cassia, eucalyptus and cumin. It has Pleasant aroma and have been used commercially in perfumes and cosmetics. The molecular weight of this colorless compound is 148.2 g/mol^{-1}(Choi, 2003).Para-cymene (Fig. 1 b) is a cyclic organic compound with the molecular formula $C_{10}H_{14}$. This compound belongs to alkyl benzene group and is related to monoterpene. Its structure contains a benzene ring with two methyl isopropyl groups. Para-cymene is insoluble in water but soluble in ethanol. The molecular weight of this colorless oil is 134/21 g/mol^{-1} (Bennett et al,1982).



(A)



(B)

Figure 1: Structure of cumin aldehyde (a) and Para-cimen (b)

Malondialdehyde is a common byproduct of lipid peroxidation. Lipid peroxidation is a well established mechanism of cell damage in plants and animals and used

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as an oxidative stress marker in cells and tissues. Dityrosine is a specific product of protein oxidation which is formed by various oxidation systems. This highly fluorescent molecule is resistant to acid hydrolysis and protease function. Evidence suggests that these compounds may have different effects at different concentrations. One of the most important biological effects of these compounds is their antioxidant activity. Therefore, it is necessary to examine its antioxidant properties at the concentration ranges that are orally enters the body. In the present study, the antioxidant properties of these compounds on rat liver were investigated in concentrations ranging from 0 to 160 µM.

MATERIALS AND METHODS

Rat Liver Tissue Culture

For this study we used Balb/c mice. The mice were all healthy adults 4 to 6 weeks old, weighing approximately 30-25g, male or female which were purchased from the Pasteur institute of Iran. The rats were killed by chloroform and their livers were removed under sterile conditions. The livers were collected and immediately washed twice with cold saline and sliced into 0.2 g pieces with sterile surgical scissors and forceps.

In this study we used DMEM (Dulbecco's modified Eagle's medium). The culture medium was supplemented with 30 mg l-asparagine, penicillin (200 U/ ml), streptomycin (200 mg/ ml) and 10% fetal calf serum with pH =7.4. The sterile marked petri dish was used for cultivation, and 5 ml of culture medium and 2.0 g liver tissue was added to each Plate. To investigate the antioxidant enzyme's activity and the biomarkers of liver oxidative damage, Para-cymene and cumin aldehyde in concentrations of 0, 10, 20, 40, 80, 120 and 160 µM were added to the cell cultures. Then the culture media were incubated at 37 ° C in a humidified atmosphere with % 5 CO_2 for 24 hours.

Preparation of tissue for biochemical analysis

After 24 hours of incubation in various concentrations of Para-cymene and cumin aldehyde all the samples were separately homogenized by Homogenizer. After the centrifugation at 10000g for10 min, the supernatant was used for determination of enzyme activity and liver tissue oxidative damage biomarkers levels in 25 ° C.

Measurement of enzyme activity

Measurement of superoxide dismutase activity (SOD)

After 24 hours of incubation, the culture medium was evaluated based on the Kono method for the determination of superoxide dismutase activity in the liver tissue. In this method, first hydroxyl amine, nitro blue Tetrazolium and Na_2CO_3 2 M was added to a test tube. Then EDTA 1/0 mM, buffer and extract was added and nitro blue Tetrazolium absorbance shift versus Blank was evaluated at 560 nm(Kono,1978).

Measurement of catalase activity (CAT)

Catalase activity in liver homogenates was measured by the decomposition of H_2O_2 at 240 nm. Tissue homogenates (0.1 mg of protein/ml) were added to one ml of reaction mixtures containing 15 mM H_2O_2, 0.1% Triton X-100, and 50mM potassium phosphate, pH 7.4. Units of enzyme activity were determined using the extinction coefficient of 0.0394 $mM^{-1}cm^{-1}$ for H_2O_2. One unit of catalase activity is defined as 1 mmol H_2O_2 consumed/ min/mg of protein (Aebi,1984).

Measuring the activity of glutathione peroxidase (GPX)

Glutathione peroxidase activity was assayed by the method of Flohe & Gunzler .liver homogenates (0.1 mg of protein/ml) were suspended in 30^oC reaction mixtures (one ml) containing 1 mM glutathione, 0.96 units/ml glutathione reductase, 100 mM EDTA, and 50 mM potassium phosphate (pH 7.4). The reaction was started by sequential addition of 150 mM NADPH and 1.2 mM **tert**-butyl hydroperoxide, and then absorbance was measured at 340 nm. Units of enzyme activity were determined using the extinction coefficient of 6.22 $mM^{-1}cm^{-1}$. One unit of the enzyme is defined as 1 nmol NADPH consumed/min/mg of protein (Flohe,1984).

Determining the of markers of oxidative damage

Evaluation the level of malondialdehyde (MDA) in liver tissue

We used wills method to determination the amount of malondialdehyde in liver tissue. In this method, a solution of tri-chloro acetic acid was mixed with extract in a test tube and incubated for 15 min in hot water bath. Then Thiobarbituric acid solution was added. Homogenized liver proteins can also react with thiobarbituric acid and produce colored species, resulting in enhanced absorption and cause lipid peroxidation to be

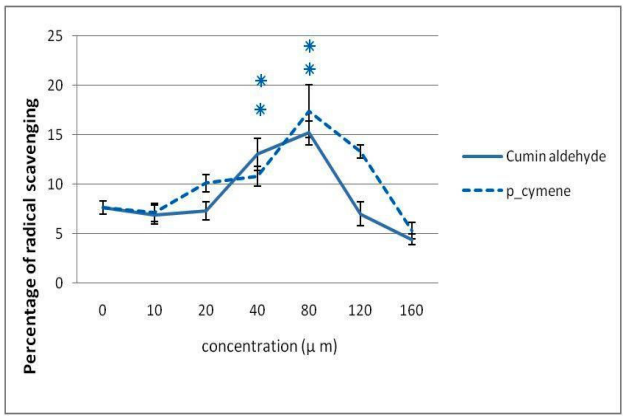
over estimated. So in the first step these proteins were removed using trichloroacetic acid. In this method, the incorporation of MDA and thiobarbituric acid under high temperature (100 -90° C) and acidic conditions led to the formation of thiobarbituric acid reactive pink substances. Then the absorption was evaluated against blank at nm 532 in spectrophotometry, and the concentration of this composition has been reported based on micromoles MDA per grams of protein (Wills,1965).

Evaluation of Dityrosine (DT) in liver

Dityrosine was detected by reverse phase HPLC and UV detector in nm 280 and combined with radiation fluorescence light (ex. 280 nm and em 410 nm). First, the extracts were mixed with trichloroacetic acid in a test tube. HPLC column was equipped with a backbone column for this analysis. Gradient of 10 mM ammonium acetate adjusted with acetic acid and methanol at pH=5.4, starting with 1% methanol, and increased to 10% in 30 min. the flow rate was 8.0 mL / min. Concentration of this compound has been reported as dityrosine micromoles per gram of protein (Amado et al,1984).

Methods and tools for data analysis

Experiments were performed in the range of 0 - 160 µM cumin aldehyde and Para-cymene and each experiment was repeated three times. The mean and standard deviation was determined for results. The ANOVA test results were analyzed using SPSS and the statistical comparisons between the results was performed ($P < 0.05$).



* Significant difference compared to the control.

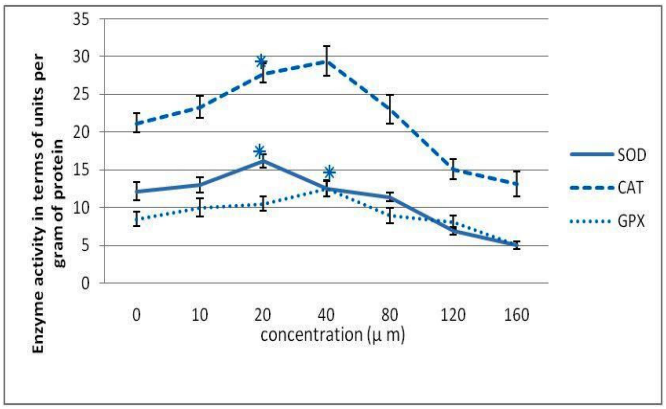
Figure 2: Percentage of radical scavenging under effect of different concentrations of cumin aldehyde and Para-cumin

RESULTS

Evaluation of antioxidant activity of cumin aldehyde and para-cymene on liver tissue

Cumin aldehyde and Para-cymene effects on the collection activity of (1, 1 – diphenyl 2-picryl hydrazyl) DPPH radicals were studied in the liver tissue. The results showed that the cumin aldehyde and para-cymene enhance the DPPH radical scavenging activity in the concentration range of 40 to 80 µM. So that the maximum inhibition of DPPH radical was in 80 µM, and show 50% and 56% significant increase compared to control respectively (Figure 2).

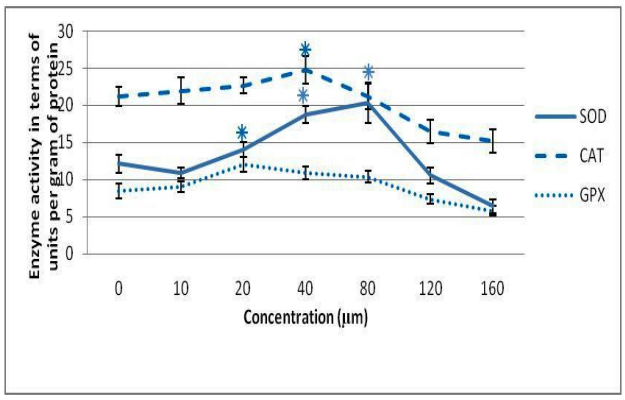
Evaluation of antioxidant enzyme activity in liver



* Significant difference compared to the control.

Figure 3: Cumin aldehyde effect on the activity of antioxidant enzymes in the liver

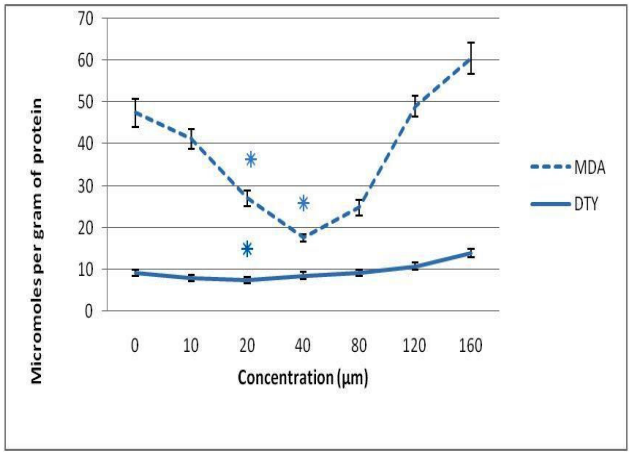
The cumin aldehyde and para-cymene effect on the antioxidant enzyme activity of liver was studied in this research. The results showed that the cumin aldehyde had significant enhancer effect at the concentration of 20 µM on SOD activity, at concentrations of 20 and 40 µM concentrations on the catalase activity, and at concentration of 40 µM on the glutathione peroxidase activity to 34% , 37% and 48% respectively (Fig. 3).



*Significant difference compared to the control

Figure 4: Paracemenu effects on antioxidant enzyme activities in miceliver

Para-cymene at concentrations of 40 and 80 µM significantly enhance superoxide dismutase activity, the catalase activity at a concentration of 40 µM, and the glutathione peroxidase activity at 20 µM to 65%, 17% and 41% compared to control respectively (Figure 4).



*Significant difference compared to the control**.**

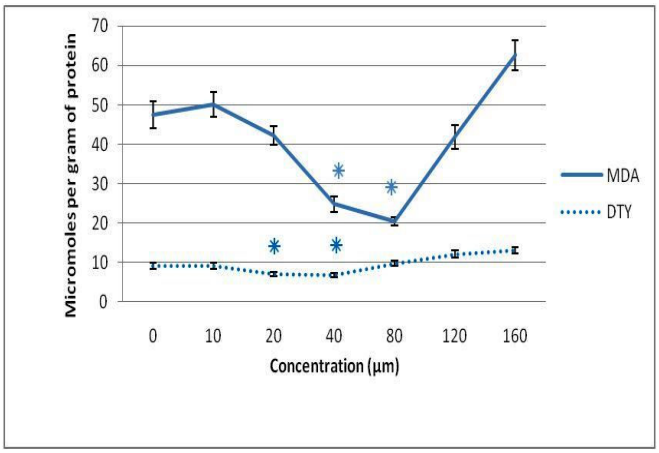
Figure 5: Effect of cumin aldehyde levels on of liver oxidative damage biomarkers

Evaluation of the level of oxidative damage biomarkers in mice liver tissue

The effect of different concentrations of cumin dialdehyde and Para-cymene on the level of oxidative damage biomarkers was evaluated in liver tissue.

The results showed that cumin aldehyde significantly reduced the damage biomarker levels at the concentrations of 20 and 40 µM, malondialdehyde at the concentration of 20 µM, and Dityrosine levels to 63% and 18% respectively compared to control (Figure 5).

Para-cymene at concentrations of 40 and 80 µM significantly reduced the malondialdehyde levels, and at concentrations of 20 and 40 µM decrease the Dityrosine level to 57% and 25% compared to control (Figure 6).



* Significant difference compared to the control

Figure 6: Para-cumin effect on the level of liver damage biomarkers

CONCLUSION

Evaluation the effect of different concentrations of cumin aldehyde and Para-cymene on antioxidant enzymes and biomarkers of liver damage in mice after 24 hours of incubation invitro

In the present study the effects of cumin aldehyde and Para-cymene in concentrations ranging from 0 to 160 µM was studied on the activity of antioxidant enzymes and biomarkers of oxidative damage on mice liver invitro. According to the data, cumin aldehyde and Para-cumin in the concentrations of 40 and 80 µM increase the DPPH scavenging activity.

Also cumin aldehyde at concentrations in the range of 20 and 40 µM, and Para-cymene in the concentration in the range of 20, 40 and 80 µM increase the activity of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase compared to control, and significantly decreased the levels of oxidative damage biomarkers such as malondialdehyde and Dityrosine compared to control.

In line with the results of our observations, further studies can be provided. Among these the research was conducted by Koppulas & et al. in 2011 showed that

the cumin extract could reduce the lipid peroxidation and increase the catalase, glutathione peroxidase and superoxide dismutase levels (Koppulas,2001)

In another study, the compounds in cumin roots, leaves and flowers were investigated by Lamiaa & et al. in 2011. The results showed that cumin flowers had phenolic compounds and essential fats which scavenge the free radicals and prevent the lipid peroxidation (Lamiaa,2011). Laboratory works conducted in 2006 revealed that cumin prevents the production of free radicals. Treatment of diabetic rats with cumin leads to decreasing the blood glucose, glycosylated hemoglobin, creatinine and blood urea within 28 days, balance the serum insulin and glycogen in liver cells and skeletal muscle cells, and significantly decrease the oxidative stress in the liver and kidney tissues. In other words, cumin improved the liver and kidney's antioxidant status of diabetic's mice (Aruna,2006).

A study was performed on the inhibition of LDL oxidation by phenolic substances in different essential oil. In this study, several different types of essential fats in the prevention of LDL oxidation invitro were examined. In this study the activity of several different types of essential fats in the prevention of LDL oxidation was examined invitro. The research was conducted in 1993 by Frankel et al. showed that the Para-cymene in cumin extract prevents the lipid peroxidation (Frankel,1993).

In a study by Surya et al. in 2005, two groups of diabetic and non-diabetic mice were injected with a mixture of juice and cumin in gastric. After 6 weeks, the level of catalase, glutathione peroxidase, superoxide dismutase, glutathione-S-transferase was increased in the liver of diabetic mice compared with nondiabetic mice, and decreased the levels of free radicals that induced oxidative stress in diabetic mice(Surya,2005)

Since the essential fats derived from cumin could induced the balance of the activity of antioxidant enzymes and because high cumin phenolic content and its antioxidant activity, this plant can be used as a good source of nutrition should be used to protect the body. Therefore, cumin aldehyde and Para-cymene can be used in certain range of concentrations, which have antioxidant activity in liver tissue in order to protect liver against ROS driven diseases.

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