FABAD J. Pharm. Sci., 27, 1-5, 2002

RESEARCH ARTICLES / BİLİMSEL ARAŞTIRMALAR

Antioxidant Effect of Cabbage (*Brassica oleracea* var. *capitata*) on Oxidative Stress in Cadmium Treated Mice

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***Antioxidant Effect of Cabbage* (Brassica oleracea var. *capitata*) On Oxidative Stress in Cadmium Treated Mice**

Summary : *In this study, the antioxidant effect of cabbage (*Brassica oleraceae var. *capitata) on cadmium-induced oxidative stress was investigated. Mice were pretreated with cabbage juice (1:1 w/w in distilled water for 7 days) prior to cadmium treatment (at a single dose, 2 mg/kg, i.p.) to provide adequate antioxidant status. Cadmium significantly increased the lipid peroxidation (LPO) in liver and lung, however no significant change was observed in plasma LPO. Animals treated with cadmium showed significant depletion of glutathione (GSH) in liver. Cabbage juice prevented the depletion of GSH significantly. It can be clearly seen that pretreatment with cabbage juice decreased the cadmium-induced LPO and increased GSH levels. We suggest that Brassicaceae vegetables such as cabbage may have antioxidant effects against heavy metal induced oxidative stress.*

Key Words: *Cadmium, cabbage, Brassica oleraceae var. capitata oxidative stress, lipid peroxidation, antioxidants, glutathione.*

Kadmiyum Uygulanan Farelerde Lahananın (*Brassica oleracea var. *capitata*) Oksidatif Stres Üzerine Antioksidan Etkisi

Özet : *Bu çalışmada, lahananın (*Brassica oleraceae var. *capitata), kadmiyumla oluşturulan oksidatif stres üzerine etkisi araştırıldı. Kadmiyum uygulamasından (tek doz, 2mg/kg, i.p.) önce, yeterli antioksidan kapasiteyi sağlamak için farelere lahana suyu (1:1 w/w, 7 gün boyunca) uygulandı. Kadmiyum karaciğer ve akciğerlerde lipid peroksidasyonu (LPO) anlamlı derecede artırırken plazma LPO'da anlamlı bir değişiklik saptanmadı. Kadmiyum uygulanan hayvanların karaciğerinde glutatyon (GSH) tüketimi de anlamlı derecede fazlaydı. Lahana suyuyla ön tedavinin kadmiyumla oluşan LPO'yu azalttığı ve GSH düzeyini artırdığı görüldü. Sonuçlarımıza göre, lahana gibi Brassicaceae sebzelerinin ağır metallerle oluşan oksidatif strese karşı antioksidan etkilerinin olabileceğini söyleyebiliriz.*

Anahtar kelimeler : *Kadmiyum, lahana, Brassica oleracea* var. *capitata, oksidatif stres, lipid peroksidasyon, antioksidanlar, glutatyon.*

Received : 1.11.2001
Revised : 16.12.2001
Accepted : 18.3.2002

INTRODUCTION

Fruits and vegetables are important sources of antioxidants as well as minerals (zinc, manganese etc.) and vitamins. Natural and artificial antioxidants may inhibit the generation of oxygen free radicals or directly scavenge the reactive oxygen free radicals. They effectively remove the excess free radicals in cells . Herbs, spices, vegetables, and fruits are among those which are believed to have antioxidant activity[1-3].

The studies indicate that Brassicaceae vegetables such as cabbage, *B. oleracea* var. *capitata* and broccoli, *B. oleracea* var. *italica* may play a special role in the treatment and prevention of some diseases in both experimental animals and human[2,4]. In addition, it was shown that some extracts isolated from vegetables can inhibit the carcinogenic effects of xeno-

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biotics[5,6]. It is understood that cabbage juice has antitumoral, antithrombotic, antidiabetic and hepatoprotective activity from the results of studies on the biological activity of *Cruciferae* plants[6,7]. Eisner showed a decreased death rate in uranium exposed rats.[8]

Recent studies have described LPO as an early and sensitive consequence of heavy metal (eg, mercury, lead, and cadmium) exposure[9,10]. Cadmium is an abundant, nonessential element that is generating concern owing to its accumulation in the environment as a result of industrial practices. Soluble cadmium salts accumulate and result in toxicity to the liver, kidneys, brain, lungs, testes, heart and central nervous system. It is well accepted that acute or chronic exposure to cadmium, by decreasing the antioxidant capacity and producing lipid peroxides in membrane, elevates free radical generation and impairs the cell functions[11,12].

Aerobic cells have evolved a complex enzymatic and non-enzymatic defence mechanism to deal with oxygen derivated radicals mediated oxidative stress. One of the principal cellular defence molecule is GSH, a non-protein cysteine reservoir in the liver involved in many cellular processes including the detoxification of endogenous and exogenous compounds and which is able to protect cellular constituents from the toxic effects of free radicals[13]. The sulfhydryl groups in its structure may effectively bind with certain divalent metals such as cadmium. It has been shown that in vitro addition of GSH ameliorates cadmium toxicity in isolated rat hepatocytes[14].

The purpose of this study was to investigate the possible modulating effect of cabbage (*Brassica oleracea* var. *capitata*) juice on cadmium-induced oxidative stress. LPO and GSH were determined as the indicators of free radical injury .

MATERIAL AND METHODS

Animals

Local breed albino male mice were purchased from the Refik Saydam Hıfzısıhha Institute, Animal Care Unit, Ankara, Turkey. The animals weighing 20-25 g were housed in group cages under normal laboratory conditions (temperature 20-22°C, natural day-night cycle), and free access to commercial chow and water. The food was withdrawn 12-16 h before the experiment.

Preparation of cabbage juice

Cabbage juice was prepared according to the previously reported method of Komatsu et al.[15]. Washed cabbage leaves were homogenized in an equal weight of distilled water in a mixer (1:1 w/w), and then centrifuged. The supernatant was filtered through filter paper.

Treatment

The mice were divided into three groups. Cabbage juice (0.1ml/10g of body weight/day for 7 days) was given to the cabbage juice+cadmium group, and an equal volume distilled water was given to the other groups (control and cadmium groups) by gastric gavages for 7 days. Cadmium (as $CdCl_2$ 2mg/kg, i.p.) was administered 1 h after the treatment of cabbage juice or distilled water on 7th day. The animals were sacrificed 24 h after the cadmium injection. The control group animals received an equal volume of the NaCl 0.9 % , i.p. The oral administration was carried out once a day at 9:00-10:00 a.m. Blood was taken intracardiacally. The liver and lungs were then removed, rinsed in ice-cold 0.9 % NaCl, blotted dry, and weighed.

Lipid peroxidation in plasma

0.1ml of plasma was added to 2.0ml of trichloroacetic acid (TCA, 15% w/v) - thiobarbituric acid (TBA, 0.375 %w/v) - 0.25 N HCl and centrifuged at 10.000 xg for 5 min. The supernatant was mixed with 20µl of butylated hydroxy toluene (BHT, 0.02% in 95% ethanol w/v) and heated for 15 min. in a boiling water bath. After cooling under tap water, it was centrifuged at 10.000 x g for 5 min. The absorbance of the plasma sample was read at 532 nm against blank. Results were expressed as nmol MDA / ml[16].

Lipid peroxidation in tissues

The method of Ohkawa et al. (1979), modified by Ja-

mall and Smith (1985), was used to determine LPO in tissues[17,18]. This method is based on the formation of a red chromophore, following the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) and other breakdown products of peroxidized lipids. The breakdown product of 1, 1, 3, 3- tetraethoxy propane (TEP) was used as standard. The results were expressed as nmol of MDA per gram of tissue. The absorbance of the supernatant was measured at 532 nm against tissue blank.

Nonprotein sulfhydryl groups (cellular GSH) in liver

200 mg of liver was homogenized in 8.0 ml of 0.02 M EDTA in an ice bath. The homogenates were kept in the ice bath until used. Aliquots of 5.0 ml of the homogenates were mixed in 15.0 ml test tubes with 4.0 ml distilled water and 1.0 ml of 50 % trichloroacetic acid (TCA). The tubes were centrifuged for 15 min. at approximately 3000 xg. 2.0 ml of supernatant was mixed with 4.0 ml of 0.4 M Tris buffer, pH 8.9, 0.1 ml Ellman's reagent [5,5`-dithiobis-(2-nitro-benzoic acid)] (DTNB) and the tubes were mixed. The absorbance was read within 5 min after the addition of DTNB at 412 nm against a reagent blank with no homogenate. Results were expressed as (µmol GSH / g tissue[19].

RESULTS AND DISCUSSION

No mortality was observed in cadmium-treated animals. When the toxic effects of cadmium have been investigated with several routes of exposure and various doses, it was shown that cadmium does not generate free radicals although it elevates LPO in various tissues, especially in the liver[20]. Manca et al. assessed the susceptibility to LPO of some organs of rats which had been given various doses of cadmium by a single dose of intraperitoneal injection[21]. The results suggested that, LPO is an early and sensitive reaction to cadmium exposure. In contrast to these findings, Jamall and Smith[22] have shown that cadmium treatment does not cause any significant increase in LPO in either liver or kidney of rat. In our study, a pronounced increase of LPO was observed in the livers of the mice that were administered a single intraperitoneal dose of cadmium compared to control ($p<0.001$).

There is little information on the nature of the mechanisms which are responsible for the generation of free radicals and increasing LPO in lung tissue following acute or chronic exposure to cadmium. Leduc et al. reported that chronic cadmium treatment can cause lung emphysema, the mechanism of which may depend on oxidant radical release by alveolar macrophages in vitro[23]. Although, there were no statistically significant differences in the plasma LPO of cadmium administered group, it was clearly seen that the LPO was higher than the control group lung tissue ($p<0.05$). Results are shown in table 1.

Table 1: LPO levels in plasma, liver and lung 24 h after intraperitoneal administration of cadmium as $CdCl_2$.

Experimental groups	Plasma MDA (nmol /ml)	Liver MDA (nmol /g wet wt)	Lung MDA (nmol /g wet wt)
Control	1.29 ± 0.06	292.30 ± 13.90	252.40 ± 12.10
Cadmium[a]	1.72 ± 0.20	506.10 ± 28.30 **	294.40 ± 13.30*
Cabbage juice + Cadmium[b]	1.43 ± 0.03	288.9 ± 10.1**	251.7± 10.30 *

Results are expressed as the mean (± SEM) of six mice per group. a: compared to control group ; b: compared to cadmium group ; *: $p< 0.05$, ** $p< 0.0001$

It was shown that in many studies, coadministration of some antioxidants such as vitamin E, ascorbic acid, or N-acetylcysteine with cadmium protected the animals against hepatic toxicity[24,25]. Also, Kyung and Fleming reported that sulfur compounds derived from cabbage have an antimicrobial activity[26]. Previous studies have shown that *Brassica oleracea* var. *capitata* includes alkaloids, proteins, lipids, resinoid substances, calcium salts, iron, magnesium oxide, various antioxidant vitamins (Vit. A and C) and especially organosulfur groups[27,28]. When the effect of cabbage juice was compared with the effect of cadmium in plasma, liver and lung , we found a significant protective effect against cadmium-induced free radical injury ($p<0.001$ in liver tissue; $p<0.05$ in lung tissue) though there was no important decrease in plasma LPO. It has been reported that cadmium-induced LPO in the liver of mice tissue has been enhanced by GSH depletion and suppressed by thiol supplementation[13,25] and, various vegetable extracts such as broccoli and green onion can induce hepatic glutathione-S-transferase activity[1,3]. Actually, we

found a pronounced decrease in hepatic GSH content in the cadmium group (Control: 11.4±0.5; Cd: 4.8±0.3 (μmol/g wet wt; $p<0.0001$). Pretreatment of cabbage juice significantly prevented the depletion of the GSH (Cabbage juice + Cd: 7.8±0.5 (μmol/g wet wt; $p<0.001$) compared to cadmium group (Fig. 1). Probably, tissue GSH content progressively increases during the treatment with cabbage juice. We therefore

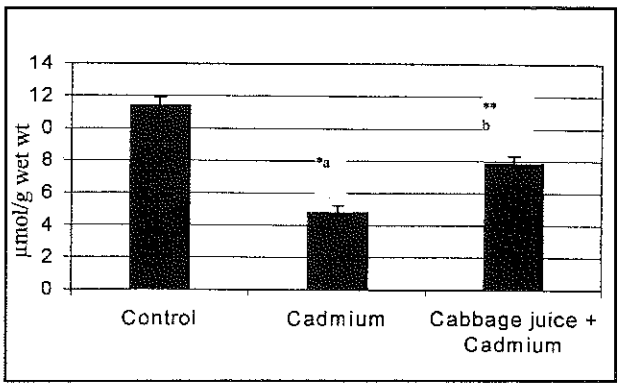

Fig. 1. GSH levels in livers 24 h after intraperitoneal administration of cadmium as $CdCl_2$. GSH: μmol/g wet wt.; a: compared to control group; b: compared to cadmium group; * $p<0.0001$; ** $p<0.001$.

evaluated the hepatic LPO and GSH levels in cadmium and cabbage juice + cadmium groups to find out whether there was a relationship between them. In the hepatic LPO level, there was a negative correlation between GSH and LPO level in the liver of the cadmium group which was extremely significant ($r = -0.9756$, $p = 0.0009$). Similarly, a very significant negative correlation was found in the same parameters in the liver of the cabbage juice administered group ($r = -0.9264$, $p = 0.0079$).

According to the present results and previous studies, consumption of some vegetables such as cabbage may have beneficial effects against some diseases and may prevent heavy metal toxicity without using any synthetic antioxidants. In addition, chemopreventive effects of cabbage juice on oxidative stress may depend on its structure, especially the sulfur groups, which can induce the synthesis of the GSH in tissues.

Statistical analysis

Data were presented as the mean ± SEM of six mice per group. The data were analyzed with the help of a computer software (Instat). Statistical differences level between groups were calculated using Student's t test.

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