Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(11)60086-5

Protection of cadmium chloride induced DNA damage by Lamiaceae plants

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ARTICLE INFO

Article history:
Received 13 March 2011
Received in revised form 2 April 2011
Accepted 23 April 2011
Available online 10 May 2011

Keywords:
Cadmium chloride
DNA damage
Lamiaceae plants
Phenols
Comet assay
Antioxidant activity

ABSTRACT

Objective: To analyze the total phenolic content, DNA protecting and radical scavenging activity of ethanolic leaf extracts of three Lamiaceae plants, *i.e. Anisomelos malabarica* (*A. malabarica*), *Leucas aspera* (*L. aspera*) and *Ocimum basilicum* (*O. basilicum*). **Methods:** The total polyphenols and flavonoids were analyzed in the ethanolic leaf extracts of the lamiaceae plants. To determine the DNA protecting activity, various concentrations of the plant extracts were prepared and treated on cultured HepG2 human lung cancer cells. The pretreated cells were exposed to H_2O_2 to induce DNA damage through oxidative stress. Comet assay was done and the tail length of individual comets was measured. Nitric oxide and superoxide anion scavenging activities of lamiaceae plants were analyzed. **Results:** Among the three plant extracts, the highest amount of total phenolic content was found in *O. basilicum* (189.33 mg/g), whereas *A. malabarica* showed high levels of flavonoids (10.66 mg/g). *O. basilicum* also showed high levels of DNA protecting (85%) and radical scavenging activity. **Conclusions:** The results of this study shows that bioactive phenols present in lamiaceae plants may prevent carcinogenesis through scavenging free radicals and inhibiting DNA damage.

1. Introduction

Reactive oxygen species are cytotoxic and disrupt the normal metabolism through oxidative damage to lipids, proteins and nucleic acids[1]. Heavy metals present in the environment are of increasing concern due to their carcinogenic effect. Cadmium, a heavy metal, widely used in industries, is considered as a human carcinogen[2]. The human body has limited capacity to respond to cadmium exposure, hence the metal cannot undergo metabolic degradation. This increases the half-time of cadmium in human body to 15 and 20 years[3]. Cadmium produces DNA single strand breaks, DNA-protein cross-links, chromosomal aberrations and changes the gene expression of proto-oncogenes[4]. The mechanism of cadmium induced toxicity was found to be the inhibition of GSH and production of radicals including nitric oxide and superoxide anion[5]. A recent study suggests that low levels of cadmium inhibit the activities of phosphatase and kinases that are involved in DNA repair[6]. Experiments on animal models and cell lines showed that cadmium is involved in the pathogenesis of cancers in organs like breast, lung, prostate and kidney[7]. Cadmium is found to be a potent toxic to sperm cells whose number and motility are reduced[8].

Plants contain a wide variety of secondary metabolites like phenols and flavonoids with antioxidant activities[9,10]. These perform functions like scavenging free radicals, donating hydrogen atoms or electrons, or chelating metal cations[11]. Lamiaceae members are found to be rich source of polyphenols, flavonoids and vitamins[12,13]. A wide variety of biological activities like anticholinesterase, antimicrobial, anti-inflammatory and antiviral activities were reported in lamiaceae members[14,15]. In the present study, lamiaceae members were tested for its anticarcinogenic effect on cadmium induced DNA damage. The plants were also tested for the scavenging of nitric oxide and superoxide anion free radicals.

2. Materials and methods

2. 1. Preparation of leaf extract

Anisomelos malabarica (*A. malabarica*), *Leucas aspera* (*L. aspera*) and *Ocimum basilicum* (*O. basilicum*) were collected

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from the Bharathiar University campus, Coimbatore, India. The leaves were shade dried, ground into fine powder and extracted with ethanol (95%) in a Soxhlet apparatus for 24 h. The extract was concentrated under reduced pressure and controlled temperature (40-50 ℃) using a rotary evaporator.

2. 2. Estimation of total phenols and flavonoids

Total phenols were expressed in gallic acid equivalents (GAE)[16]. The dry extract was diluted in water and 100 mL of this solution were transferred to a 10 mL volumetric flask, to which 0.5 mL undiluted Folin-Ciocalteu reagent was added. After 1 min, 1.5 mL 20% (w/v) Na_2CO_3 were added and the volume was made up to 10 mL with H_2O. After 1 h incubation at 25 ℃, the absorbance was measured at 760 nm and compared with a pre-prepared gallic acid calibration curve. Flavonoids in the plant extracts were measured as described by Chang *et al*[17]. An aliquot of 500 μL of plant extract was dissolved in 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride hexahydrate, 0.1 mL potassium acetate and 2.8 mL of water. The above contents were incubated at room temperature for 40 min. The absorbance was read at 415 nm using a UV-Vis spectrophotometer. Quercetin was used as a standard and the results were expressed as milligram quercetin equivalents.

2.3. Cell culture and treatment

Human lung cancer HepG2 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% L-glutamine. After reaching 80% confluence, the cells were incubated with different concentrations of *A. malabarica*, *L. aspera* and *O. basilicum* leaf extract individually for 4 h at 37 ℃. The pretreated cells were exposed to 150 μM/mL of $CdCl_2$ for 1 h. The cells were washed with PBS and harvested using trypsin/EDTA. The cell suspension was centrifuged at 1 500 rpm for 5 min. The supernatant was discarded and the cell pellet was resuspended in PBS.

2.4. Analysis of DNA damage using comet assay

Comet assay was performed as descried by Singh *et al*[18]. The cell suspension was mixed with 75 μL of 0.5% low melting agarose and layered on the slides precoated with 1% normal melting agarose. After solidification of agarose, 75 μL of 0.5% low melting agarose was layered. The slides were immersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris and 1% Sodium laurylasarcosine; 1% Triton X-100 and 10% DMSO) for 1 h at 4 ℃. The slides were then placed in an electrophoresis tank filled with 300 mM NaOH and 10 mM Na_2EDTA (pH 13) for 40 min. The slides were electrophoresed at 25 V/300mA for 20 min at 4 ℃. After electrophoresis, the slides were washed with neutralizing buffer (0.4 M Tris, pH 7.5) for 5 min at 4 ℃ and treated with ethanol for 5 min. The slides were dried and stained with 50 μL of ethidium bromide(20 μg/mL) and viewed under Nikon fluorescent microscope with a 515-560 nm excitation filter. Comets like structures were seen in cells with DNA damage. The tail length and head diameter of the comet structures were measured using an ocular micrometer. The DNA damage was quantified as comet tail length (μm) = (maximum total length) - (head diameter)[19].

2.5. Analysis of nitric oxide

The HepG2 cells were cultured and treated with plant extracts and CdCl2 as described above. Nitric oxide was measured in the supernatant of culture medium using Griess reagent. 50 μL of supernatant of culture medium was mixed with equal amount of Griess reagent and incubated for 10 min. The absorbance was read at 550 nm using a spectrophotometer. Cells treated with cadmium chloride were used as controls.

2.6. Analysis of superoxide anion

The superoixde anion was measured using nitroblue tetrazolium (NBT) as described by Siwik *et al*[20]. After treatment with the plant extracts and cadmium chloride, the cells were incubated in PBS containing 100 μM/L of NBT for 90 min. The cells were harvested and centrifuged at 10 000 rpm for 10 min. The cell pellet was resuspended in 100 μM/L of pyridine and heated at 80 ℃ for 90 min. The purple color formed by formazan was measured at 540 nm using UV-Vis spectrophotometer. The amount of NBT reduced to formazan was used to calculate the levels of superoxide anion.

2. . Statistical analysis

All the values were expressed as mean ± SD. Students *'t'* test was performed to analyze significance between groups using SPSS software. A value of $P<0.05$ was considered as significant level.

3. Results

The phenolic content of the three plants, *O. basilicum*, *L. aspera* and *A. malabarica* ranged from 141 mg to 189 mg per gram of dry weight. High levels of phenols were found in *O. basilicum* leaf extract (Table 1). The anticarcinogenic activity of three plant extracts was tested using comet assay. The cells pretreated with *A. malabarica*, *L. aspera* and *O. basilicum* leaf extracts showed DNA protective effect in a dose dependent manner. Among the three plants, *O. basilicum* showed a maximum DNA protecting activity. When compared with controls, the *O. basilicum* extracts treated cells showed a significant ($P<0.05$) decrease in comet tail length. At 350 μg/mL this plant extract protected 85% of DNA from cadmium toxicity. *L. aspera* and *A. malabarica* also protected the cells from cadmium toxicity. At 350 μg/mL, 55% and 70% of DNA was protected by *L. aspera* and *A. malabarica* (Figure 1). When compared with other two members, the amount of total phenol content was highest in *O. bascillicum* leaf extract and further it showed significant DNA protecting ability.

Table 1
Total phenols and flavonoids of three lamiaceae plants (Mean ± SD).

Plants	Total phenols (mg/g gallic acid equivalent)	Flavonoids (mg/g quercetin equivalent)
A. malabarica	176.33 ± 4.16	10.66 ± 1.52
L. aspera	141.33 ± 2.51	7.66 ± 1.15
O. basilicum	189.33 ± 1.52	8.33 ± 0.57

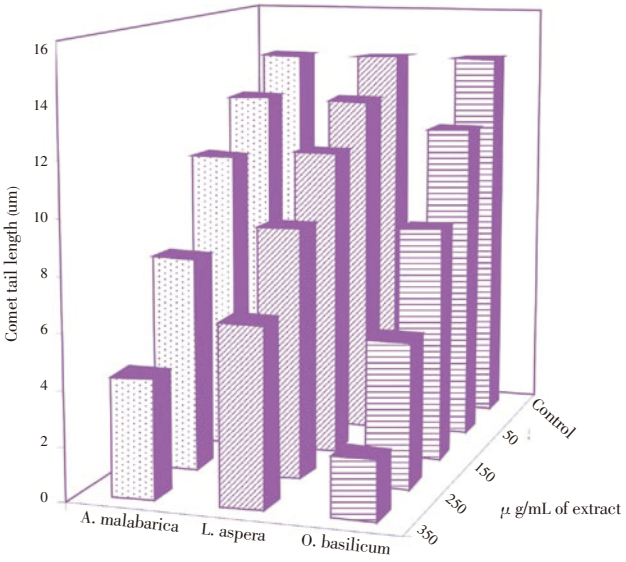

Figure 1. DNA protective effect of lamiaceae plants on cadmium chloride induced genotoxicity.
* denotes that data significantly (P<0.05) different from the control.

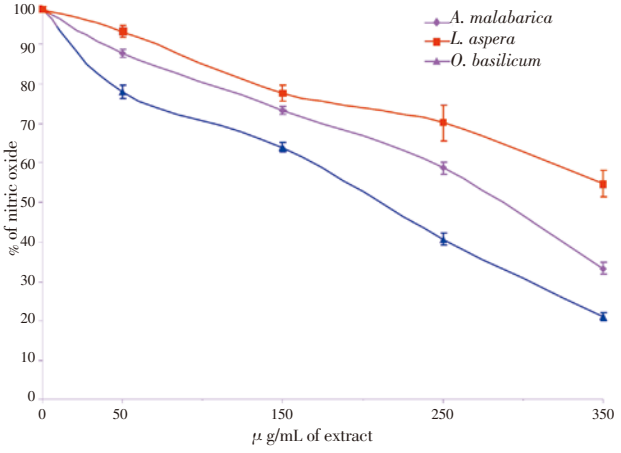

Figure 2. Nitric oxide scavenging effect of three lamiaceae plants.
* denotes that data significantly (P<0.05) different from the control.

The nitric oxide generated by cadmium chloride was measured using Griess reagent. Nitric oxide generated in cells treated with 150 μg/mL of cadmium chloride was considered as 100%. The cells pretreated with the three plant extracts showed a dose dependent inhibition of nitric oxide. The maximal nitric oxide inhibitory effect was exhibited by *O. basilicum* which inhibited 79% of nitric oxide at 350 μg/mL of extract. An inhibition of 67% and 45% of nitric oxide was observed in cells treated with *A. malabarica* and *L. aspera* extracts (Figure 2). The presence of superoxide anion was analyzed using the NBT. A statistically significant inhibition of superoxide was observed in all concentration of the three plants. At 350 μg/mL, *O. basilicum* inhibited 78% of superoxide anion produced by cadmium chloride, whereas *L. aspera* and *A. malabarica* inhibited 64% and 46% of nitric oxide (Figure 3). Among the three plants tested, *O. basilicum* contains higher polyphenols and flavonoids than *L. aspera* and *A. malabarica*. It also exhibited maximal levels of DNA protecting and free radical scavenging activity against cadmium chloride induced toxicity.

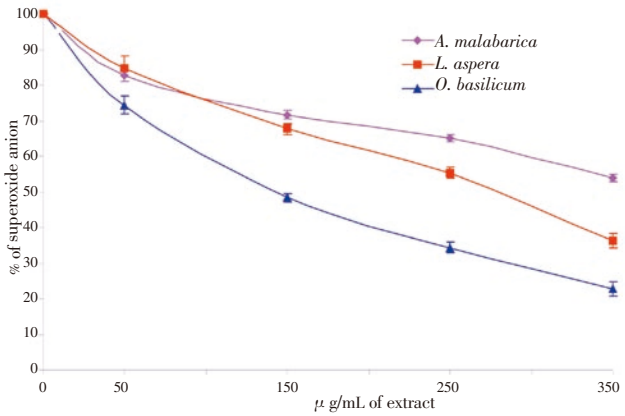

Figure 3. Super oxide anion scavenging effect of three lamiaceae plants.
* denotes that data significantly (P<0.05) different from the control.

4. Discussion

DNA damage by free radicals is an important contributor for cancer development. Cadmium is an occupational and environmental pollutant that has been associated with renal, skeletal, vascular, reproductive and respiratory disorders[21]. Cd-induced oxidative stress has been associated with production of reactive oxygen species consisting mainly of superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radicals (OH^-)[22]. These reactive oxygen species play a crucial role in the development of tissue damage in various human diseases such as cancer, aging, neurodegenerative disease, malaria and arteriosclerosis[23,24]. Epidemiological studies suggest that people exposed to cadmium have an increased risk of bladder and prostate cancer[25,26].

Medicinal plants and herbs are promising and diverse sources of natural antioxidants. Most of the members of Lamiaceae family have been found to be very effective with regard to natural antioxidants[27-29]. Phenolic compounds like flavonoids from *L. aspera* showed significant DPPH radical scavenging activity[30-33]. A linear positive relationship existed between the antioxidant activity and total phenolic content of the *O. bascillicum*[34]. In the present study the anticarcinogenic potential exhibited by *A. malabarica*, *L. aspera* and *O. bascillicum* may be due to the presence of high levels of phenols. Free radical scavenging activity of phenolic compounds may act as potential anticarcinogens. According to Aherne *et al*[35], *Rosmarinus officinalis*, *Origanum vulgare* and *Salvia officinalis* showed significant antioxidant activity and DNA protecting ability which may be due to the presence of phenols. The antioxidant activity of phenols is mainly due to their redox properties, which allow

them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In the present study, *O. bascillicum* showed high level of phenols and exhibited DNA protecting activity against cadmium chloride induced genotoxicity. This plant also showed maximal free radical scavenging activity when compared with the other two plants tested in this study. The findings of the present study suggest that the proper use of herbal products is safer and may provide beneficial health effects to humans.

Conflict of interest

We declare that have no conflict of interest.

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