South African Journal of Botany 102 (2016) 18–25

Contents lists available at ScienceDirect

South African Journal of Botany

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

Genotoxic and antigenotoxic potential of the aqueous leaf extracts of *Amaranthus spinosus* Linn. using *Allium cepa* assay

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ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 19 June 2015
Accepted 24 June 2015
Available online 27 August 2015

Edited by L Verschaeve

Keywords:
Allium cepa
Amaranthus spinosus
Antigenotoxicity
Genotoxicity
H_2O_2

ABSTRACT

The aim of the present study was to evaluate the possible genotoxic effects of *A. spinosus* leaf extracts on *Allium cepa* root meristematic cells and its antigenotoxic effects against H_2O_2-induced genetic damage in *A. cepa*. The excessive reduction in mitotic index (MI) (A4, 3 h, 23.37 ± 1.41) and extremely significant ($P < 0.001$) levels of clastogenicity (A4, 3 h, 98.14 ± 0.70) was observed after genotoxicity assay. The clastogenic abnormalities observed include bizarre nucleus, chromosome bridges, cytomixis, etc. In antigenotoxicity studies, initial toxicity was induced by two concentrations (7% and 3%) of H_2O_2. Higher percentage of nuclear lesions in treatment with H2O2 (99.27 ± 0.19) and its significant reduction after modulatory treatment (5 mg/L; 32.25 ± 7.69) was observed in the results, and indicates the chemopreventive activity of the plant extract at a critical concentration (5 mg/L). Here, the extent of antimutagenicity at this specific concentration (5 mg/L) was strong as the percentage of inhibition was greater than 40% (67.51 ± 8.68). The results demonstrated that the aqueous plant extract of *A. spinosus* have the ability to inhibit the oxidative damage induced by the direct-acting mutagen (H_2O_2) at a precise concentration.

1. Introduction

Amaranthus spinosus (Family: Amaranthaceae), is a spinous and noxious weed, used as a tribal medicine for a wide range of diseases and also as a vegetable. In Ayurveda, the plant is used as a digestive, laxative, diuretic, stomachic, antipyretic, improves the appetite and biliousness. It cures blood diseases, leprosy, bronchitis, piles and leucorrhoea (Kirtikar and Basu, 2001). The plant extract possesses severe effect on hematology (Olufemi et al., 2003). It shows immunomodulatory (Tatiya et al., 2007), anthelmintic (Assiak et al., 2002), analgesic (Krishnamurthi et al., 2010), antidiabetic, antihyperlipidemic, spermatogenic (Girija and Lakshman, 2011; Sangameswaran and Jayakar, 2008), hepatoprotective (Zeashan et al., 2009, 2008), anticancer (Cristine et al., 2013), antioxidant (Odhavo et al., 2007; Zeashan et al., 2009) and chemoprotective activities (Kumar et al., 2010).

A. spinosus has several active constituents like alkaloids, flavonoids, glycosides, phenolic acids, steroids, terpenoids, saponins, betalains, b-sitosterol, stigmasterol, rutin, catechuic tannins, etc. Betalains in the stem bark of *A. spinosus* were identified as amaranthine, isoamaranthine, hydroxycinnamates, quercetin and kaempferol glycosides (Srinivasan et al., 2005; Ibewuike et al., 1997; Rastogi and Mehrotra, 1999; Stintzing et al., 2004; Hilou et al., 2006). It also contains amaranthoside, a lignan glycoside, amaricin, a coumaroyl adenosine along with stigmasterol glycoside, betaine such as glycinebetaine and trigonelline (Azhar-ul-Haq et al., 2006; Blunden et al., 1999). Betalains are well-known for their antioxidant, anticancer, antiviral and antiparasitosis activities (Kapadia et al., 1995, 1996; Patkai et al., 1997).

Induction of DNA damage, especially oxidative damage, is a crucial step in the development of cancer and other degenerative processes such as cardiovascular and neurodegenerative diseases as well as premature ageing (Finkel and Holbrook, 2000; Wiseman et al., 1995). Moreover, several studies have shown that *Amaranthus* extracts are strong free radical scavengers (Kumar et al., 2011; Odhavo et al., 2007). Chemical compounds of medicinal herbs exert antioxidant and free radical scavenging effect either separately or in synergistic ways (Romero-Jimenez et al., 2005). Identification and characterization of the active principles may lead to viable strategies to reduce the risk for cancer in humans (Dearfield et al., 2002). Plants and their byproducts contain biologically active compounds that are used as traditional medicine (Aggarwal and Shishodia, 2006). Intake of diet with a high amount of vegetables, fruits and other plant-based foods can reduce the risk of cancer and chronic diseases (Schaefer, 2002).

A. spinosus exposed to automobile emission was studied for the antioxidant capacity by Singh and Dahiya (2002). *A. spinosus* was shown to possess a very good free radical scavenging system for combating air pollution through analysis of the enzyme superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and phenolic peroxidase activity. It was reported that Amaranthaceae plants contain betalain pigments which showed strong antioxidant activity by the DPPH assay. The leaves of *Amaranthus* are a potential alternative source of betalains because of their betacyanin pigments. They also show

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http://dx.doi.org/10.1016/j.sajb.2015.06.018
0254-6299/

anticancer activity (Singh and Singh, 2011). Hence, the antioxidant activity of *A. spinosus* extract may be due to its betalain content (Singh et al., 1997).

The use of medicinal plant extracts for the treatment of human diseases is an ancient practice which has greatly increased in recent years (Oyeyemi and Bakare, 2013). The secondary metabolism of higher plants has been shown to be an almost inexhaustible source of compounds with possible biological activity (Santos et al., 2006). The use of antimutagens and anticarcinogens in everyday life has been suggested to be an effective procedure for preventing human cancer and genetic diseases (Ferguson, 1994). Higher plants used extensively in traditional medicines are increasingly being screened for their role in modulating the activity of environmental genotoxicants (Sreeranjini and Siril, 2011). Medicinal plants contain bioactive compounds which can act to block or reverse carcinogenesis at early stages (Lippman et al., 1994).

The present investigation was planned to study the genotoxic and antigenotoxic activity of different concentrations of *A. spinosus* aqueous extract using *A. cepa* test system. The antimutagenic study was conducted by assessing the activity of plant extract at lower concentrations against DNA damage induced by reactive oxygen species (ROS) after treatment with H_2O_2. H_2O_2 itself acts as an oxidizing agent that induces oxidative stress (Torbergesen and Collins, 2000) and therefore *A. cepa*-based antigenotoxicity test is considered an easily adoptable method for screening plants for their antigenotoxic potential.

2. Materials and methods

2.1. Collection of plant materials

Fresh plant materials of *A. spinosus* Linn. were collected from Calicut University campus in Malappuram district of Kerala (latitude, 10°7′34″ N; longitude, 75°53′25″E; altitude, 45–50 m). The plant was identified taxonomically and voucher specimen herbarized (CALI 123743). For the *Allium cepa* assay, certified bulbs were purchased from an agricultural vendor.

2.2. Genotoxicity assay

Fresh aqueous leaf extracts of *A. spinosus* were prepared with the help of mortar and pestle. To make the stock solution, 1 g of leaf was weighed and ground in 100 ml distilled water. The lowest concentrations of the extract viz., 0.05 g/L (A_1), 0.1 g/L (A_2), 0.5 g/L (A_3) and 1 g/L (A_4) were prepared for toxicity analysis by diluting the stock solution with distilled water. Uniform-sized healthy bulbs of *A. cepa* were sorted and planted in sterilized sandy soil without manure to prevent cellular alterations. Germinated bulbs with healthy roots (1–2 cm) were collected at peak mitotic period (9 am–10 am) and washed in distilled water. The bases of onion bulbs bearing roots were suspended in extract solutions. Onion bulbs treated with distilled water were taken as controls. After treatment for various time durations, a few healthy root tips excised from each bulb were washed thoroughly with distilled water and immediately fixed in ethanol/glacial acetic acid (2:1) fixative (modified Carnoy's fluid) for 1 h. After hydrolysis in 1 N HCl for 15 min at room temperature, mitotic squash preparations were made with improved techniques (Sharma and Sharma, 1980) using 2% acetocarmine.

Two slides were made for each treatment and scoring was done from five sites that were randomly selected to determine the mitotic index (MI) and the percentage of chromosomal aberrations. The MI was calculated for each treatment as the number of cells in mitosis/total number of cells counted and expressed as percentage. The cells were also scored for cytological abnormalities and the percentage of chromosomal aberrations. It was determined as the ratio of number of aberrant cells to the total number of cells observed. About 152–309 cells were scanned, tabulated and photomicrographs were taken with Olympus Camedia C-4000 zoom digital camera. The most frequent abnormalities are shown in photomicrographs.

2.3. Antigenotoxicity assay

In the antigenotoxicity test, the inhibition of mutagenic activity of H_2O_2 by the test sample (*A. spinosus* extract) was determined by analyzing H_2O_2-induced chromosomal aberrations in the root tip of *A. cepa* in the presence of the test sample. For this assay, healthy onion bulbs were carefully unscaled and the old roots were removed. They were then placed on top of small jars containing distilled water and were allowed to germinate in the dark for 48 h at room temperature (25 ± 2 °C). To make the test sample, 1 g of leaf (*A. spinosus*) was weighed and ground in 100 ml distilled water. Lowest concentrations of the extract viz., 0.5 mg/L, 1 mg/L, 5 mg/L and 10 mg/L were prepared for antigenotoxicity analysis by diluting the stock solution with distilled water. Two concentrations of H_2O_2 (3% and 7%) were also prepared.

2.3.1. Treatment with H_2O_2

The roots germinated in water were treated with the respective concentrations (3% and 7%) of H_2O_2 for 1 h. After treatment, the roots were removed and their cytological studies were carried out as described previously. All slides were scanned, tabulated and photomicrographs were taken. The cells were scored for cytological abnormalities induced by H_2O_2.

2.3.2. Modulatory treatment with plant extracts

The bulbs treated with H_2O_2 were washed thoroughly in distilled water and treated with respective concentrations of *A. spinosus* extract for 24 h (0.5 mg/L, 1 mg/L, 5 mg/L and 10 mg/L). The root tips were excised and their cytological studies were carried out as described previously. About 267–289 dividing cells from five fields were scored for each treatment.

The cells were scored for cytological aberrations existing after the modulatory treatment with plant extracts. Inhibitory activity of the chromosomal aberrations induced by H_2O_2 is confirmed by calculating the percentage of inhibition. The antigenotoxic potential of aqueous extract of *A. spinosus* was calculated using the formula:

$$\text{Inhibitory activity } (\%) = \text{A}-\text{B} \div \text{A}-\text{C} \times 100$$

A: Number of aberrant cells induced by H_2O_2
B: Number of aberrant cells induced by H_2O_2 after modulatory treatment
C: Number of aberrant cells induced in the negative control (distilled water)

Root tips treated with 3% and 7% H_2O_2 transferred to distilled water was used as positive control (PC). Root tips treated with distilled water alone are used as negative control (NC). All experiments were made in triplicate.

2.4. Statistical analysis

Data obtained from both genotoxicity and antigenotoxicity assays were subjected to statistical analysis. Duncan's multiple range test and one-way ANOVA was performed to determine mean separation and significance of treatments using SPSS version 20, SPSS Inc., Chicago, USA.

3. Results

3.1. Genotoxicity assay

The results obtained reflect the genotoxic and antigenotoxic activities of *A. spinosus* on *A. cepa* root tip cells. Acute toxicity was observed