

Antimutagenic effects of ethanolic extracts of *Amaranthus spinosus* and *Amaranthus hybridus*.

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Abstract: Several compounds of plant origin like Ascorbic acid, ellagic acid and tannins are known to be associated with anti mutagenic activity. Mechanism of mutagenesis is complex and different in different mutagens and thus antimutagenesis process is also varied. During present investigation protective effects of *A. spinosus* and *A. hybridus* ethanolic extracts exhibit concentration dependent effect of EMS induced toxicity against *S. typhimurium*. The 95% of survival *S. typhimurium* at concentration of 8 mg/plate of ethanolic extract of *A. spinosus* and *A. hybridus* caused 96% survival of *S. typhimurium* against EMS induced toxicity at 8 mg/plate concentration.

Keywords: *Amaranthus spinosus*; Ethanolic extract; Antimutagenic assay.

1. Introduction

Plants synthesize an array of structurally and functionally diverse bioactive secondary metabolites. The phytochemicals are subject to wide experimental scrutiny for their pharmacological and therapeutic potential. Substantial work has been reported on the screening of medicinal and edible plants for their mutagenicity and/or antimutagenic properties. Most of these natural products are regarded as potential sources of novel therapeutic agents against mutational disorders in humans.[1]

Ethyl methane sulphonate is a direct acting environmental mutagen, which can react directly with DNA and cause alkylation leading to the inhibition and or stimulation of specific gene expression. Plants harbor compounds, which protect cells from mutagen-induced damage. Mechanism of action of the antimutagenic agents present in plants is not very clear. Those compounds which act inside the cell and modify the response of the cell to a mutagen are called as antimutagenic. Those that act on the mutagens outside the cells and inactivate its mutagenicity are called as desmutagenic agents. During present investigation the protective effect of *Amaranthus spinosus* and *Amaranthus hybridus* extracts were tested against EMS induced toxicity in *Salmonella typhimurium*. [1]

2. Materials

Plant materials

Amaranthus spinosus (VTJ 30, BSI/SRC/5/23/09-10/Tech-956) and *Amaranthus hybridus* (VTJ 36, BSI/SRC/5/23/09-10/Tech-934) were collected and identified by using standard flora and also authenticated by Botanical Survey of India, Pune. The collected leaves were separated and washed several times with distilled water to remove foreign material then it is air dried for 24 h. Thereafter, they were dried in oven at 25°C for 7 days. The dried leaves and seeds were powdered using mechanical grinder and stored in schott bottles until use. [2]

Culture: A Standard strain of *Salmonella typhimurium* (ATCC 2501) was purchased from National chemical Laboratory, Pune. The culture was maintained on Nutrient broth slants at 37°C. Ethyl methyl sulphonate was purchased from Sigma-Aldrich Pvt Ltd, Mumbai-India

3. Methods

Toxicity of the Extracts against *Salmonella typhimurium*:

Toxicity of the extracts of 10g of leaf powder of *A. spinosus* and *A. hybridus* were tested against *Salmonella typhimurium* by agar dilution method. Nutrient agar was autoclaved and different concentrations of the extracts (0, 0.5, 1.0, 1.5 mg/ml) of all selected medicinal plants were added separately to it at 45°C-50°C temperature, aseptically. Twenty ml of these media was poured in each sterile petri plates. After solidification, the plates were inoculated with 50µl of 24 hours old culture of *Salmonella typhimurium* culture in nutrient broth containing approximately 2×10^4 cfu/ml. Three plates were used for each concentration. The plates were incubated at 37°C for 24 hours. Number of colonies appeared were counted and compared with that of control.

Antimutagenic Activity

The protective effect of selected medicinal plants extracts against EMS induced toxicity in *Salmonella typhimurium* was tested. The dose of Ethyl Methane sulphonate was determined by testing various concentrations and it was found that 1µl EMS killed about 75% of organisms and thus EMS was used as a 1µl plate. Twenty ml of autoclaved nutrient agar was poured in each sterile petriplate and was allowed to solidify. The 50µl of 24 hr-old broth culture of *S. typhimurium* was taken in a test tube containing 50µl of phosphate buffer. To these tubes various concentrations of extracts (0, 2, 4, 6 and 8 mg in separate tubes) and 1µl of EMS were added aseptically. Tubes were incubated for thirty minutes at 37°C and after incubation; 2ml of molten top agar was added to each tube and poured into the solidified petri plates. Plates were kept in triplicates for each concentration and incubated for 24 hours at 37±1°C.

Numbers of surviving colonies were counted and percent of survival were calculated by comparing with the control [3].

4. Results

A protective effect of *A. spinosus* and *A. hybridus* ethanolic extracts exhibit concentration dependent protective effect against EMS induced toxicity.

The 95% of survival *S.typhimurium* at concentration of 8 mg/plate of ethanolic extract of *A.spininosus* which was followed by 41% of 2 mg/plate, 37% of 6 mg/plate, 32% at 4mg/plate and 20% survival of 0 mg/plate respectively (Table No.1).

The maximum survival of *S.typhimurium* was found at 8 mg/plate concentration, while 34%, 31%, 34% & 18% survival has concentration range of 6, 4, 2 & 0 mg/plate respectively (Table No.2)

Table No.1 Protective effect of ethanol extracts of *A.spininosus* against EMS in *Salmonella typhimurium* strain. (\pm Standard deviation)

Plant	Extract mg/plate	Control no EMS	EMS 1 μ L/ml	Percentage survival
Control	0	271 \pm 17.0	62 \pm 10.0	20
<i>A.spininosus</i>	2	135 \pm 10.0	62 \pm 10.0	41
<i>A.spininosus</i>	4	321 \pm 26.0	107 \pm 13.0	32
<i>A.spininosus</i>	6	287 \pm 25.0	111 \pm 17.0	37
<i>A.spininosus</i>	8	242 \pm 24.0	230 \pm 21.0	94

Table No.2. Protective effect of ethanol extract of *A.hybridus* against EMS in *S. typhimurium* strain (\pm Standard deviation).

Plant	Extract mg/plate	Control no EMS	EMS 1 μ L/ml	Percentage survival
Control	0	261 \pm 17.0	58 \pm 9.0	18
<i>A.hybridus</i>	2	134 \pm 10.0	54 \pm 9.0	34
<i>A.hybridus</i>	4	287 \pm 24.0	104 \pm 12.0	31
<i>A.hybridus</i>	6	289 \pm 25.0	98 \pm 16.0	34
<i>A.hybridus</i>	8	221 \pm 22.0	234 \pm 22.0	96

5. Discussion

During present investigation protective effects of *A. spinosus* and *A. hybridus*, ethanolic extracts exhibit concentration dependent protective effect against EMS induced toxicity against *S. typhimurium*. *A.aspera* caused about 91% followed by *A.spininosus* 95%, and *A.hybridus* 96%. *A.hybridus* causes maximum survival of *S. typhimurium* against EMS induced toxicity at 8 mg/plate concentration. (Table no.1&2). These results were agreed with studied that the total methanol extract of the leaves of *A.aspera* has antimutagenic activity. [4]

The variations in the antimutagenic activity in these plant extract might be due to the differences in the active constituents and their combinations in the extract. [5] Present findings are in agreements of other workers who have reported concentration dependent antimutagenic activity in other plants. [6] In studied plants the significant antimutagenic activity was observed against direct acting mutagens suggesting that these extracts may protect DNA damage from mutagen. However, the inhibition of mutagenesis is often complex, acting through multiple mechanisms.

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