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Antimutagenic Activities of Methanol Extracts of Some Endemic *Astragalus* Species

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The present study was conducted to evaluate the antimutagenic activity of methanol extracts of *Astragalus campylosema*, *A. cristianus, A. globosus* and *A. lineatus* plants. The possible antimutagenic potential of different species was examined against mutagens sodium azide (NaN_3), 9-aminoacridine (9AC) and 4-nitro-1-quinoline oxide (4NQO) in *Salmonella typhimurium* strains TA1535-TA1537 and TA1538 using standard plate incorporation assay. In order to assess antimutagenic effects of *Astragalus* species. The plants methanol extracts were done at the doses of 5, 0.5 and 0.05 µg/plate. *Astragalus* species tested have different inhibition rate on mutagenicity at the concentration between 0.05-5 µg/plate doses. The best activity was obtained from *A. cristianus* aerial and root extracts, respectively; 78 % (5 µg/plate); 64 % (0.5 µg/plate), 79 % (5 µg/plate); 40 % (0.5 µg/plate) on TA1538.

Key Words: ***Astragallus*, Methanol extract, Antimutagenic activity, AMES, *Salmonella*/microsome test.**

INTRODUCTION

in vivo and *in vitro* studies showed that some natural compounds which are obtained from the fruit, leaf and roots of the plants have regulator roles on coenobitic effects[1]. The identification and characterization of the antimutagenic and anticarcinogenic effects of these compounds are important strategy for the treatment of cancer and many other human associated diseases. Still some bioactive compounds and their derivatives are observed that inhibiting the carcinogenesis in experimental systems which include beginning,

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development and/or spreading phases. Therefore, there has been considerable interest in antimutagenicity of several secondary plant metabolites[2-6].

To determine the antimutagenic activities of plant metabolites with *in vivo* studies by using experiment animals is not preferred beginning test system because of high cost and long time requirement[7]. Thus, many scientists searched alternative bioassay methods to reduce time requirement and cost for anticarsinogenity studies[8]. AMES-*Salmonelle*/microsome test system is one of the important *in vitro* tests which is commonly used for determining antimutagen/anticarsinogens or on the contrary mutagen/carsinogens[9] extracted from plant species.

Astragalus L. is the largest genus of flowering plants, containing up to 3000 species[10]. This genus is a member of the legume family (Fabaceae) and traditionally classified in the tribe Galageae. *Astragalus* is also the largest genus in Turkey where it is represented by 400 species in 62 sections[11]. *Astragalus* species have been commonly used in Traditional Chinese Medicine for thousands of years. It contains antioxidants, which protect cells against damage of free radicals[12-16]. *Astragalus* has antiviral properties and stimulates the immune system, preventing colds and upper respiratory infections as well as to lower blood pressure and treat diabetes. Furthermore, *Astragalus* has antibacterial, antiinflammatory and diuretic activities. In the previous studies, saponin, formononetin, calycosin, astragoloside and calycosin-7-O-β-D-glucoside isolated from the *Astragallus* species were reported as antioxidant agent against superoxide anion and hydroxyl radical[12-16]. In addition, isoflavons, 1-[(3R)-7,8-dimethoxybenzopyranyl]-4-hydroxybenzoquinone (astragaluquinone) and (3S)-7,1'-dihydroxy-8,3'-dimethoxy-isoflavan (8-methoxyvestitol), 7-hydroxy-2',3',4'-trimethoxyisoflavan molecules isolated from *Astragallus* species were known to have antimicrobial activities[17,18].

However, to the best of our knowledge, there have been no attempts to evaluate the antimutagenic activities of *Astragalus* species until now. Therefore, the aim of this study was to investigate the antimutagenic activity of *Astragalus* species.

EXPERIMENTAL

Plant samples of four endemic *Astragalus* species including *A. cristianus, A. campylosema, A. globosus* and *A. lineatus* were collected from different locations in the vicinity of Erzurum, located in the eastern Anatolia, Turkey (Table-1). The taxonomic identification of plant materials was confirmed by a plant taxonomist, Dr. Meryem Sengul, in Department of Biology, Atatürk University, Erzurum, Turkey. Collected plant materials were dried in the shade and the leaves of plant were separated from the stem and ground in a grinder with a 2 mm in diameter mesh.

TABLE-1
Astragalus SPECIES TESTED

Plants	Herbarium number of the vouchers	Locality	Status	Altitude (m)
Astragalus campylosema Boiss. ssp. *campylosema*	9817	Ilica, Erzurum	End	1780
Astragalus cristianus L.	9816	Askale, Erzurum	End	1910
Astragalus globosus Vahl	9820	Palandöken, Erzurum	End	2200
Astragalus lineatus Lam.	9819	Çat, Erzurum	End	1820

Test chemicals: Mutagens, direct acting mutagens sodium azide (NaN_3), 4-nitro-1-quinoline oksit (4-NQO), 9-aminoacridine (9AC) were obtained from Sigma-Aldrich Co (St. Louis, MO, USA).

Preparation of the methanol extract: The dried and powdered root (100 g) and aerial parts (100 g) of plants were extracted with 1 L of methanol using a Soxhlet extractor (ISOPAD, Heidelberg, Germany) for 72 h at a temperature not exceeding the boiling point of the solvent[19]. The extract was filtered using Whatmann filter paper (No. 1) and then concentrated *in vacuo* at 40 °C using a rotary evaporator (Buchi Labortechnic AG, Flawil, Switzerland). The extract was then lyophilized and kept in the dark at 4 °C until tested.

Bacterial strains: *Salmonella typhimurium* strains TA1535 (ATCC® Number: 29629), TA1537 (ATCC® Number: 29630) and TA1538 (ATCC® Number: 29631) were obtained from The American Type Culture Collection-Georgetown University, Bacteria Department, Washington, USA. For all assays, fresh cultures were prepared from frozen permanent cultures and incubated and shaken overnight at 37 ºC to a concentration of *ca.* $1\text{-}2 \times 10^9$ bacteria/mL.

Antimutagenicity assay: Antimutagenicity assay was performed according to Mortelmans and Zeiger[8] following the procedure reported by Maron and Ames[20] with modifications. Suitable concentrations of known mutagens for *S. typhimurium* strains were chosen from the linear part of a concentration-response curve: 4-nitro-1-quinoline oxide (4NQO in DMSO 0.2 µg/plate) for TA1538, sodium azide (NaN_3 in distilled water 1 µg/plate) for TA 1535, 9-aminoacridine (9AC in methanol 125 µg/plate) for TA1537. 100 µL of the overnight bacterial culture, 50 µL mutagens, 50 µL test compounds at different concentrations (0.05, 0.5, 5 µg/plate in 10 % DMSO) and 500 µL phosphate buffer were added to 2 mL of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose plates. Histidine independent revertant colonies and viable cells were scored on plates after incubation at 37 ºC for 48 or 72 h. The experiments were

repeated at least twice and each concentration determined in triplicate. 4NQO for TA1538, NaN_3 for TA1535, 9AC for TA1537 were used as positive reference. The solvent control % 10 DMSO was used as negative control. After incubation, revertant colonies were counted and the percentage inhibition assay calculated: [1-T/M] × 100, where T is the revertant per plate in the presence of mutagen and plant extract and M is the revertants per plate in the control (10 % DMSO, no plant extract). A non-antimutagenic effect was considered to give a value smaller than 25 %, a moderate effect a value between 25-40 % and strong antimutagenicity a value greater than 40 %[5].

Statistical analysis: The results are presented as the average and standard error of three experiments with triplicate plates/dose experiment. The data were further analyzed for statistical significance using analysis of variance (ANOVA) and the difference among means was compared by high-range statistical domain using Tukey's test. A level of probability < 0.05 was taken as indicating statistical significance.

RESULTS AND DISCUSSION

The possible antimutagenic potential of different *Astragalus* species were examined against 4-NQO, NaN_3 and 9-AC in *S. typhimurium* strains TA1535-TA1537 and TA1538 using standard plate incorporation assay. In order to assess antimutagenic effects of Astragalus species induction or suppression of revertant colonies was examined in *S. typhimurium* strains TA1535-TA1537-TA1538. In the assay; plant methanol extract were done at the doses of 5, 0.5 and 0.05 μg/plate, the results are shown in Table-2 (Figs. 1-3).

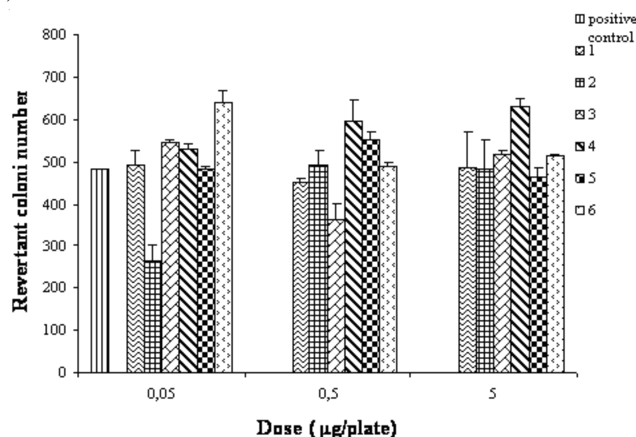

Fig. 1. Number of revertant colonies of *S. typhimurium* TA1535 applied to 1 = *A. cristianus* (aerial part); 2 = *A. cristianus* (root); 3 = *A. campylosema* (aerial part); 4 = *A. campylosema* (root); 5 = *A. lineatus* (aerial part); 6 = *A. globosus* (aerial part). Points represent the mean number of revertant colonies ± standard error. DMSO, dimethylsulfoxide, was used as negative control; NaN_3, sodium azide, was used as positive control

TABLE-2
ANTIMUTAGENICITY OF THE EXTRACTS OBTAINED FROM *Astragalus* SPECIES AGAINST *Salmonella typhimurium* TA1535, TA1537 AND TA1538

Test items	Concentration (µg\plate)	Revertant colonies number					
		Salmonella typhimurium TA 1535		*Salmonella typhimurium* TA 1537		*Salmonella typhimurium* TA 1538	
		Mean ± SE	Inhibition (%)	Mean ± SE	Inhibition (%)	Mean ± SE	Inhibition (%)
DMSO*	-	18.66 ± 1.76		16.33 ± 2.60		8.66 ± 2.51	
4NQO†	0.2					491.33 ± 48.33	
NaN_3†	1.0	483.33 ± 0.64					
9AC†				347.66 ± 37.03			
Astragalus campylosema (aerial part)	5.00	516.33 ± 11.66	-	427.33 ± 35.57	-	540.33 ± 18.66	-
	0.50	360.00 ± 40.52	25	288.66 ± 23.96	-	474.67 ± 16.28	-
	0.05	544.33 ± 6.35	-	483.33 ± 8.81	-	432.33 ± 29.03	-
Astragalus campylosema (root)	5.00	631.66 ± 17.63	-	268.66 ± 6.33	22	137.66 ± 8.41	72
	0.50	597.00 ± 48.12	-	172.66 ± 6.35	50	224.00 ± 39.00	54
	0.05	530.66 ± 12.91	-	179.66 ± 15.30	48	592.66 ± 62.63	-
Astragalus cristianus (aerial part)	5.00	487.66 ± 84.73	-	254.00 ± 3.05	27	103.33 ± 11.79	78
	0.50	451.00 ± 8.71	-	266.66 ± 12.01	23	175.33 ± 12.25	64
	0.05	493.33 ± 34.26	-	253.33 ± 26.03	27	401.66 ± 30.86	-
Astragalus cristianus (root)	5.00	483.67 ± 67.55	-	318.00 ± 32.51	-	109.00 ± 8.32	79
	0.50	491.66 ± 35.89	-	213.33 ± 12.01	39	296.00 ± 20.13	40
	0.05	264.66 ± 37.03	-	404.66 ± 4.80	-	556.00 ± 8.32	-
Astragalus globosus (aerial part)	5.00	514.33 ± 4.66	-	369.33 ± 13.22	-	602.66 ± 5.81	-
	0.50	490.00 ± 8.88	-	374.33 ± 14.46	-	528.00 ± 18.04	-
	0.05	640.66 ± 27.16	-	376.33 ± 12.49	-	425.33 ± 13.13	-
Astragalus lineatus (aerial part)	5.00	463.66 ± 22.55	-	596.66 ± 21.42	-	259.00 ± 4.61	47
	0.50	551.99 ± 19.36	-	451.66 ± 8.41	-	387.00 ± 70.23	21
	0.05	484.33 ± 6.08	-	414.33 ± 12.41	-	452.33 ± 43.66	-

Points represent the mean number of revertant colonies ± standard error (SE); *DMSO (dimethylsulfoxide) was used as negative control.
†4NQO (4-nitroquinoline-1-oxide), 9AC (9-aminoacridine) and NaN_3 (sodium azide) were used as positive control for *S. typhimurium* TA1538, TA1537 and TA1535, respectively.

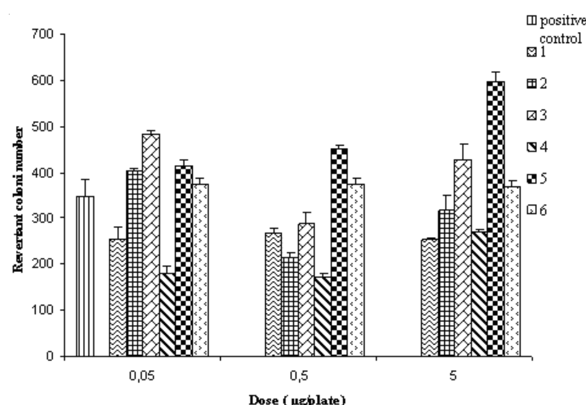

Fig. 2. Number of revertant colonies of *S. typhimurium* TA1537 applied to 1 = *A. cristianus* (aerial part); 2 = *A. cristianus* (root); 3 = *A. campylosema* (aerial part); 4 = *A. campylosema* (root); 5 = *A. lineatus* (aerial part); 6 = *A. globosus* (aerial part). Points represent the mean number of revertant colonies ± standard error. DMSO, dimethylsulfoxide, was used as negative control; 9AC, 9-aminoacridine, was used as positive control

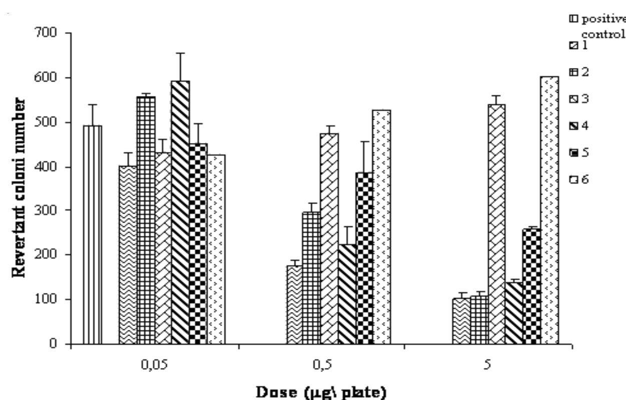

Fig. 3. Number of revertant colonies of *S. typhimurium* TA1538 applied to 1 = *A. cristianus* (aerial part); 2 = *A. cristianus* (root); 3 = *A. campylosema* (aerial part); 4 = *A. campylosema* (root); 5 = *A. lineatus* (aerial part); 6 = *A. globosus* (aerial part). Points represent the mean number of revertant colonies ± standard error. DMSO, dimethylsulfoxide, was used as negative control; 4NQO, 4-nitroquinoline-1-oxide, was used as positive control

On *S. typhimurium* TA1535 strain *A. campylosema* (aerial part) showed moderately antimutagenic activity only on 0.5 µg/plate dose with 25 % inhibition, with none of the other doses and on none of the other strains it showed activity. Besides, root extract showed strong antimutagenic activity on TA1538 strain, respectively 72-54 % with 0.5-5 µg/plate doses. The inhibition rates of the same extract's antimutagenic activity on TA 1537 strain was determined as; 22 % (5 µg/plate), 50 % (0.5 µg/plate), 48 % (0.05 µg/plate).

One of the studied endemic *Astragalus* species, *A. cristianus* plant's neither aerial nor root extracts showed antimutagenic activity on TA 1535 strain. Besides, both of the extracts from two parts of the same plant, showed strong antimutagenic activity with 5-0.5 μg/plate doses on TA 1538 strain. There are no considerable differences between inhibition rates. The inhibition rates of aerial and root parts are respectively; 78 % (5 μg/plate); 64 % (0.5 μg/plate), 79 % (5 μg/plate); 40 % (0.5 μg/plate) on TA1538. By the study with the same plant's aerial part extract on TA1537 strain, moderate antimutagenic activity was determined with three doses, which inhibition rates, respectively; 27-23-27 %. But the extract from root part showed moderate antimutagenic activity with 39 % inhibition rate at only 0.5 μg/plate dose.

On the study with *A. lineatus* species, it was determined strong antimutagenic activity with 47 % inhibition rate at 5 μg/plate doses and moderate antimutagenic activity with 27 % inhibition rate at 0.5 μg/plate dose only on TA1538 strain. *A. globosus* plant didn't show antimutagenic activity on none of strains and doses.

Salmonella/microsome assay was used to evaluate antimutagenicity activities of methanol extract of *Astragalus* species. The data indicated that *Astragalus* species have antimutagenic properties which significantly increase the inhibition percentage of mutagenicity effect on three different *Salmonella* mutant strains as dose dependent manner (Table-2).

The methanol extract of *Astragalus* species assessed by *Salmonella*/ microsome assay to evaluate antimutagenicity, Inhibition effect changed dose dependent; while each of the doses showed important antimutagenic activity (Table-2).

This is the first data about the antimutagenic activity of *Astragalus* species. Mechanism of this antimutagenic activity may be due to its antioxidant activity suggested by several other works[21-24]. The antimutagenic activity also might be related to on its ability to change membrane lipids and permeability of ion channels as suggested by others[24-26].

According to these results, the activity degree of the components can be examined by illustrating the chemical components of the plant extract and adding functional group to the isolated agent matter. The antimutagenic activities of extracts result from not only the capacity that can effect the enzymatic activation process but also having the characteristics that can collect the free radicals. The protective mechanism of various compounds that have structural differences can be multifactorial. Because of this reason the mechanism of plant originated natural compounds which have antimutagenic effect on human being, can be illustrated with *in vivo* studies. As related to these results with the detailed studies of plant originated compounds it can be possible to change them into more useful products for human being.

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