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Biological Activity and Antimutagencity of Water Soluble Phytotoxins from *Artemisia herba alba* ASSO

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Abstract: Fruit aqueous extract of *Artemisia herba alba* was studied for their effect on germination and seedling growth and for their antimutagenecity potentials. Germination percentage and total seedling length of *A. herba alba* reduced as the concentration of the aqueous fruit extract increased in both mature and immature. The toxicity of immature fruit aqueous extract against germination and seedling growth was stronger in comparison with that of mature. Moreover the inhibition of root length was more obvious than shoot length in both mature and immature fruit aqueous extract. In mutagenicity test, fruit aqueous extract of *A. herba alba* exhibited an anti-mutagenic activity. The percentage of anti-mutagenic increased with increasing aqueous extract concentration in both mature and immature fruit in the presence and absence of S9 mix. Anti-mutagenicity percentage was more obvious in immature fruit aqueous extract and in the present of S9 mix. The percentages of mutagenicity varied with fruit maturity and there was strong correlation between them. This relationship was coupled with the results of germination and seedling growth *A. herba alba.*

Key words: Germination, seedling growth, mutagenicity, phenolics, mature and immature fruit, aqueous extract

INTRODUCTION

Artemisia herba alba ASSO is a perennial shrub in desert and semi-deserts of the Middle East (Zohary, 1973) and its one of the medicinal plants that is used by bedouins (Al-Shamaony *et al*., 1994). Medicinal plants may contain bioactive compounds that posses anti-mutagenic activity (Marnewick *et al*., 2000; Tepsuwan *et al*., 1999) thereby may have a protective effect that reduce the onset of certain cancer.

Artemisia herba alba seed is a single fruit, that has mucilaginous cells which contain phytotoxic chemicals (Al-Charchafchi *et al*., 1987). These chemicals were toxic to the germination and seedling growth of other plants (Modallal and Al-Charchafchi, 2006) or even autotoxic (Al-Charchafchi *et al*., 1987). Many phytotoxic compounds like phenolics were isolated from the aqueous plant extracts (An *et al*., 2000).

Anticancer properties of some medicinal plants components are documented (Kusamran *et al*., 1998; Tepsuwan *et al*., 1999). *Salmonella* mutagenicity assay has been used to investigate the mutagenicity potential of many compounds by various means (Choi *et al*., 1996; Ikken *et al*., 1999). Plant phenolics have the ability to protect against mutagenicity and reduce genotoxicity of *Salmonella typhimurium* (Stagos *et al*., 2004; Belicova *et al*., 2001). Fruit phytochemicals have been isolated and identified as agents that block different stages in the carcinogensis process (Huang *et al*., 1994). These compounds may be interesting candidate class of chemopreventive compounds including antimutagenic and anticarcinogenic (Cardador-Martinez *et al*., 2002; Chlopcikova *et al*., 2004). In the present study, the biological activities of water soluble phytotoxins from mature and immature *A. herba alba* fruit on germination, growth and mutagenicity were investigated. At the same time, AMES test was utilized to study the antimutagenic activity of *A. herba alba* fruit aqueous extracts against *Salmonella*.

MATERIALS AND METHODS

Plant material: Fruits of *A. herba alba* were harvested from the medicinal and aromatic plant garden at Hashemite University in Jordan. Plant material was shade dried then ground and kept in glass jar at 5°C until use. Seeds of *A. heba alba* were harvested at maturity stage from the same garden and husked seeds were used in germination and seedling growth tests.

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Extracts preparation: Hundred grams of dried powder from each mature and immature fruits of *A. herba alba* were extracted with one liter of boiled distilled water in a glass jar for 24 h at 20°C. The mixture has been shaken every 1 h. The mixture was placed in boiled water bath for 1 h, centrifuged at 6000 rpm for 15 min and filtered through micro-filter Millipore (0.2 μm). The filtrate designated as full strength (100%) and stored in a freezer (-50°C) prior to use for bioassay.

Germination and seedling growth test: Fifty husked seed of *A. herba alba* were germinated in petri dishes on Whatman filter paper with 5 mL of the aqueous extracts or distilled water as control. Three replicates were incubated in a randomized complete block design at 20°C in an incubator with fluorescent light. Germination criteria were the emergence of the radical through the pericarp. Germinated seeds were recorded and total seedling length was measured after 7 days of incubation. Total seedling length was measured using five seedlings taken randomly from each dish after seven days of incubation.

Antimutagenic activity test: Ames *Salmonella* Plates pre incubation assay was used to evaluate mutagenic activity (Gichner *et al*., 1994). All samples were tested in *S. typhimurium* strain TA100 with and without S9 mix (Putnam *et al*., 1999). The S9 mix was prepared from rats as previously described by Maron and Ames (1983). Mutagenicity for *A. herba alba* water extracts from mature and immature fruits was analyzed using H_2O_2 as a standard mutagen. Plates incubated for 48-72 h at 37°C. Plates were read using Artek colony counter. Positive and negative controls were carried out in addition to the test samples. Triplicate plates were made for each test.

The number of his + revertants (after substracting the spontaneous reversions) induced by H_2O_2 tested without extract were given 100% mutagenicity. The presence of remaining revertant in the presence of mature and immature extracts was calculated accordingly.

Statistical analysis: Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS 9.0.0; SPSS Inc, Chicago, Illinois, United States of America, 1998). Paired t-test was used to test the significance between means. Statistical significance was set at $p < 0.05$.

RESULTS

Germination and seedling growth: Germination percentage of *Artemisia herba alba* seeds were significantly decreased ($p<0.05$) as the concentration of the aqueous fruits extract (mature and immature) increased. The inhibition of germination was more pronounced at concentration above 12.5% (Table 1), while complete inhibition of seed germination was occurred at 75 and 100% of the full strength extract. Immature fruit extract showed higher inhibition in germination ($p<0.05$) as compared to mature fruit extract.

The total seedling length of *A. herba alba* also decreased with increasing concentration of aqueous fruit extract as indicated in Table 2. But no significant differences were shown between mature and immature fruit extract on the inhibition of total seedling length except in 25%. Therefore it is worthwhile to investigate which plant part (root or shoot) was mainly inhibited by crude aqueous fruit extracts; an experiment was administrated that determined the effect of mature and immature aqueous fruit extract on shoot and root length (Table 3 and 4, respectively). Both extracts had significant toxic inhibitors on root and shoot length, but the toxicity on the root length was higher than shoot.

Table 1: Effect of different concentration of A. *herba alba* fruit (mature and immature) aqueous extract on its germination percentage

Concentration of full strength extract (%)	Germination percentage	
	Immature	Mature
0	86	86
6.35	74	80
12.5	50	72
25	12	36
50	0	8
75	0	0
100	0	0

Table 2: Effect of different concentration of *A. herba alba* fruit (mature and immature) aqueous extract on its total seedling length

Concentration of full strength extract (%)	Total seedling length (mm)	
	Immature	Mature
0	34.6	34.6
6.35	10.6	11.6
12.5	8.8	9.2
25	1.6	8.4
50	0.0	1.4
75	0.0	0.0
100	0.0	0.0

Table 3: Effect of different concentrations of *A. herba alba* mature fruit aqueous extract on its shoot and root length

Concentration of full strength extract (%)	Shoot length (mm)	Root length (mm)
0	5.8	28.8
6.35	5.6	5.0
12.5	5.4	3.4
25	1.4	0.2
50	0.0	0.0
75	0.0	0.0
100	0.0	0.0

Table 4: Effect of different concentrations of *A. herba alba* immature fruit aqueous extract on its shoot and root length

Concentration of full strength extract (%)	Shoot length (mm)	Root length (mm)
0	5.8	28.8
6.35	4.8	7.0
12.5	4.4	4.8
25	4.4	4.0
50	1.4	0.0
75	0.0	0.0
100	0.0	0.0

Table 5: Effect of mature and immature extracts of *A. herba alba* on the mutagenicity of H_2O_2 towards *Salmonella typhimurium* TA100 in the presence of S9 mix. Results are mean±SE from five repeats in each group

Amount of fruit extract (mg plate^{-1})	Mutagenicity of mature extract (%)	Mutagenicity of immature extract (%)
0.1	88.7±4.9	76.8±3.7
0.2	60.8±3.8	49.1±3.5
0.3	46.0±3.5	20.1±4.9
0.4	18.2±3.1	11.9±3.3
0.5	6.3±4.6	0.0

Table 6: Effect of mature and immature extracts of *A. herba alba* on the mutagenicity of H_2O_2 towards *Salmonella typhimurium* TA100 in the absence of S9 mix. Results are mean±SE from five repeats in each group

Amount of fruit extract (mg plate^{-1})	Mutagenicity of mature extract (%)	Mutagenicity of immature extract (%)
0.1	88.7±4.3	79.0±3.9
0.2	78.6±4.0	70.1±3.5
0.3	74.7±3.3	59.5±4.6
0.4	69.0±4.8	51.8±3.8
0.5	63.4±4.6	41.6±3.8

Antimutagenic activity test: Ames assay was conducted with and without S9 mix on mature and immature fruit aqueous extract of *A. herba alba* to evaluate their antimutagenicity potentials against H_2O_2 using *S. typhiumurium* strain TA100 are shown in Tables 5 and 6, respectively. Both mature and immature aqueous extracts exhibit anti-mutagenic activity against H_2O_2 at doses ≤0.5 mg plate^{-1}. Antimutagenicity percentages increase with increasing aqueous extract concentration of *A. herba alba* mature and immature fruit, but immature extract exhibit stronger anti-mutagenicity in presence and absence of S9 mix.

DISCUSSION

Artemisia herba alba fruit aqueous extracts exerted toxic effect on its germination and seedling growth. Thereby *A. herba alba* fruit extract may contain some toxic substances that reduced germination and seedling growth of its own (autotoxic) and other plant species such as *Anabasis setifera* (Modallal and Al-Charchafchi, 2006). Previous investigation reported that aqueous extract of some plant species contain phenolics or other toxic substances (Belicova *et al*., 2001). These phenolics inhibit the germination process (Blum *et al*., 1984; Al-Charchafchi *et al*., 1987), by their interference with indol acetic acid metabolism, or synthesis of protein and ion uptake by the plants (Castro *et al*., 1984). Therefore, *A. herba alba* might release some soluble phenolics to the environment which has a growth inhibitory effect on new seeding of *A. herba alba* or other plant species. This interpretation was in consistence with that reported by Modallal and Al-Charchafchi (2006) and Xu *et al*. (2003).

Moreover the fruit aqueous extracts of *A. herba alba* capable of inhibiting its root length more than shoot. This may be due to the direct contact between the root and phytotoxic compounds of the aqueous extracts which in turn may inhibit cell division (Rietjens and Alink, 2003) which is highly active in meristimatic tissue in the growing root.

The toxicity of the immature fruit aqueous extract on germination and seedling length was higher than mature which might be related to the difference in the amount and kinds of water soluble phytotoxins that released from fruit (Bewley and Black, 1998).

Both mature and immature extracts exhibit antimutagenic activity against H_2O_2 at doses ≤0.5 mg plate^{-1}. Antimutagenicity percentage increases with increasing aqueous extract concentration of *A. herba alba* in both mature and immature. It has been mentioned earlier that phenolics reduce genotoxicity (Belicova *et al*., 2001), prevent early atherosclerotic lesions (Auger *et al*., 2004), chemoprotective against toxicity (Chlopcikova *et al*., 2004), inhibit cancer cell growth (Saleem *et al*., 2002) and exhibit antimutagenic activity (Malaveille *et al*., 1998; Stich *et al*., 1982). *A. herba alba* antimutagenicity may be due to presence of phenolic compounds that may have a role in modulation of mitogenic signaling regulation and induction of G1 arrest and apoptosis (Agarwal, 2000; Kampa, 2000), or an important role against DNA damage an mutations induced by reactive oxygen species (Stagos *et al*., 2004). The antimutagenicity may be polar in nature because both mature and immature extracts were extractable in water and exhibit antimutagenic activity. Higher antimutagenicity effect was shown in the presence of S9 mix, therefore suggested that these antimutagens may interact with some specific enzymes in the liver homogenates, which is necessary for activation of chemical mutagen (Tepsuwan *et al*., 1999).

This study showed that antimutagenicity was higher in immature fruit aqueous extract compared to mature. This might be due to higher phenolic concentration in immature fruit aqueous extract compared to mature (Modallal and Al-Charchafchi, 2006) or due to differences

in kind of phenolics that were isolated from the fruit (Connor *et al*., 2002).

The results from this study suggest that toxicity of *A. herba alba* on germination, seedling growth was correlated with the total phenolic contents in the aqueous fruit extract. Phenolic compounds are well known potential phytotoxins (Al- Charchafchi *et al*., 1987) and exist as free forms, esters, or as glycosides when combined with sugars, that may be indirectly related to chemicals that is finally responsible for the observed toxic effect on germination and seedling growth (Seal *et al*., 2004). At the same time these phenolics exhibit some potential antimutagenic activity in presence or absence of S9 mix.

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REFERENCES

Agarwal, C., 2000. Anticarcinogenic effect of a polyphenolic fraction isolated from in human prostate carcinoma DU145 cells, modulation of mitogenic signaling regulators and induction of G1 arrest and apoptosis. Mol. Carcinogenic., 28: 129-138.

Al-Charchafchi, F.M.R., F.M.J. Redh and W.M. Kamal, 1987. Dormancy of *Artemisia herba alba* seeds in relation to endogenous chemical constituents. J. Biol. Sci. Res., Baghdad/Iraq, 18: 1-12.

Al-Shamaony, L., S.M. Al-khazraji and H.A. Twaij, 1994. Hypoglycaemic effect of *Artemisia herba alba*. J. Ethanopharmacol., 43: 167-171.

An, M., T. Haig and J.E. Pratley, 2000. Phytotoxicity of *Vulpia* residues II. Separation, identification and quntitation of allelochemicals from *Vulpia myuros*. J. Chem. Ecol., 26: 1465-1476.

Auger, C., P. Gerain, F. Laurent-Bichon, K. Portet, A. Bornet, B. Caporiccio, G. Cros, P.L. Teissedre and J.M. Rouanet, 2004. Phenolic from commercialized grape extracts prevent early atherosclerotic lesions in hamsters by mechanisms other than antioxidant effect. J. Agric. Food Chem., 529: 5297-5302.

Belicova, A., L. Krizkova, M. Nagy, J. Krajcovic and L. Ebringer, 2001. Phenolic acids reduce the genotoxicity of acrdine orange and ofloxacin in *Salmonella typhurium*. Folia Microbiol. (Praha), 46: 511-514.

Bewley, J.D. and M. Black, 1998. Seeds Physiology of Development and Germination (2nd Edn.). Plenum Press, New York and London.

Blum, U., B.R. Dalton and J.O. Rawling, 1984. Effects of ferulic acid and some of its microbial products on radical growth of cucumber. J. Chem. Ecol., 10: 1169-1191.

Cardador-Martinez, A., G. Loarca-Pina and B.D. Oomah, 2002. Antioxidant activity in common beans (*Phaseolus vulgaris* L.), 50: 6975-6980.

Castro, P.R.C., J.D. Rodrigues, J.C. Rabelo, R.F.A. Viega, G.P.P. Lima, P. Jureidini and I.M. Denbandal, 1984. Allelopathic action of some weed extracts on rice (*Oryza sativa* L.), Agric. Luiz de Queiro, 41: 369-381.

Chlopcikova, S., J. Psotova, P. Miketova and V. Simanek, 2004. Chemoprotective effect of plant phenolics against anthracycline-induced toxicity on rat cardiomyocytes. Phytother. Res., 18: 107-110.

Choi, M.J., J.W. Lee and B.M. Lee, 1996. Comparative assessment of DNA adduct formation. Salmonella mutagenicity and chromosome aberration assays as a short-term test for DNA damage. J. Toxic Environ. Health, 49: 271-284.

Connor, A.M., J.J. Luby, J.F. Hancock, S. Berkheimer and E.J. Hanson, 2002. Changes in the fruit antioxidant activity among blueberry cultivars during cold-temperature storage. J. Agric. Food Chem., 50: 893-898.

Gichner, T., G. Voutsinas, A. Patrineli, A. Kappas and M.J. Plfwa, 1994. Antimutagenicity of three isomers of aminobenzoic acid in *Salmonella typhimurium*. Mut. Res., 309: 201-210.

Huang, M.T., T. Ferraro and C.T. Ho, 1994. Cancer Chemoprevention by Phytochemicals in Fruits and Vegetables. An Overview In: Food Phytochemicals for Cancer Prevention I. Fruit and Vegetables (Hung, M.T.) (Eds.).

Ikken, Y., P. Morales, A. Martinez, M.L. Marin, A.I. Haza and M.I. Cambero, 1999. Antimutagenic effect of fruit and vegetable extracts against N-nitrosamines evaluated by the AMES test. J. Agric. Food Chem., 477: 3257-3264.

Kampa, M., 2000. Wine antioxidant polyphenols inhibits the proliferation of human cancer cell lines. Nutr. Cancer, 37: 223- 233.

Kusamran, W.R., A. Tepsuwan and P. Kupradinun, 1998. Antimutagenic and anticarcinogenic potentials of some Thai vegetables. Mutat. Res., 402: 247-258.

Malaveille, C., A. Hautefeuille, B. Pignatelli, G. Talaska, P. Vineis and H. Bartsch, 1998. Antimutagenic dietary phenolics as antigenotoxic substances in urothelium of smokers. Mut. Res., 402: 219-224.

Marnewick, J.L., W.C.A. Gelderblom and E. Joubert, 2000. An investigation on the antimutagenic properties of South Africa herbal teas. Mut. Res., 471: 157-166.

Maron, D. and B.N. Ames, 1983. Revised methods for the *Salmonellsa mutagenicity* test. Mut. Res., 113: 173-215.

Modallal, N.M. and F.M.R. Al-Charchafchi, 2006. Allelopathic effect of *Artemisia herba alba* on germination and seedling growth of *Anabasis setifera*. Pak. J. Biol. Sci. (In Press).

Putnam, K.P., D.W. Bombick, J.T. Avalos and D.J. Doolittle, 1999. Comparison of the cytotoxic and mutagenic potential of liquid smoke food flavorings, cigarette smoke condensate and wood smoke condensate. Food Chem. Toxicol., 37: 1113-1118.

Rietjens, I.M. and G.M. Alink, 2003. Nutrition and health-toxic substances in food. Ned Tijdschr Geneeskd, 147: 2365-2370.

Saleem, A., N.M. Husheem, P. Harkonen and K. Pihlaja, 2002. Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* retz. Fruit J. Ethnopharmacol., 8: 327-336.

Seal, A.N., T. Haig and J.E. Pratley, 2004. Evaluation of putative allochemicals in rice root exudates for their role in the suppression of arrowhead root growth. J. Chem. Ecol., 30: 1663-1678.

Stagos, D., S. Kouris and D. Kouretas, 2004. Plant phenolics protect from bleomycin-induced oxidative stress and mutagenecity in *Salmonella typhimurium* TA102. Anti. Cancer Res., 24: 743-745.

Stich, H.F., M.P. Rosin and L. Bryson, 1982. Inhibition of mutagenecity of a model nitrosation reaction by naturally occurring phenolics, coffee and tea. Mut. Res., 95: 119-128.

Tepsuwan, A., P. Kupradinun and W.R. Kusamran, 1999. Effect of *Siamese cassia* leaves on the activities of chemical carcinogen metabolizing enzymes and on mammary gland carcinogenesis in the rat. Mut. Res., 428: 369-373.

Xu, Z., D. Guo, L. Yu, M. Zhao, X. Zhang, D. Li, K. Zheng, and Y. Ye, 2003. Molecular Biological study on the action mechanism of rice allochemicals against weed. Ying Yong Sheng Tai Xue Bao, 14: 829-833.

Zohary, M., 1973. Goebotanical Foundation of the Middle East, 1st Edn. Gustraw Fischer Swets and Zeitlinger, Amesterdam, Vol. 1 and 2.