

COMPARATIVE ANALYSIS OF ACETONIC AND WATERY POLLEN EXTRACT OF *AMBROSIA ARTEMISIIFOLIA* L. ON *DROSOPHILA MELANOGASTER*

Sanja Matić, Snežana Stanić, Slavica Solujić - Sukdolak & Tanja Milošević

*Institute of Biology and Ecology, Faculty of Science, University of Kragujevac
Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia
e-mail: msmaticsanja@yahoo.com*

(Received April 15, 2008)

ABSTRACT: Chemical components of the acetone and watery extract of *Ambrosia artemisiifolia* (LINNAEUS, 1753) plant, and the two identified lactones in the acetone extract: the ambrosin and dihydroambrosin, was tested for genotoxicity activities.

The genotoxicity of the extract was tested on the eukaryotic model system *Drosophila melanogaster* using the sex-linked recessive lethal test (SLRL test). The results presented here illustrate that the two identified lactones, ambrosin and dihydroambrosin from acetone extract and same chemical substance from watery extract induce recessive lethal mutations on X-chromosome of *Drosophila melanogaster* in II and III broods, based on which we can conclude that spermatides and spermatocytes represent a more sensitive stages of spermatogenesis.

Key words: *Ambrosia artemisiifolia*, extract, genotoxicity.

INTRODUCTION

The *Ambrosia* (Asteraceae) genus is classified as a part of the Heliantheae tribe. The *Ambrosia artemisiifolia* (L.) plant is an invasive allergenic plant that produces large amounts of pollen (WANG *et al.*, 2006).

Sesquiterpene lactones ambrosin, isabelin, psilostachyn (BLOSZYK *et al.*, 1992; RUGUTT *et al.*, 2001), cumanin and peruvyn (PARKHOMENKO *et al.*, 2005; DAVID *et al.*, 1999), triterpenoids of type α - and β -amyrine and derivatives of caffeic acid have all been identified in the *Ambrosia artemisiifolia* (L.) plant (WANG *et al.*, 2006; TAMURA *et al.*, 2004). The allergenic factor of *Ambrosia* is in the pollen it contains (WAN *et al.*, 2002; TAO & HE, 2005). Different ragweed proteins have been identified in pollen extracts, some of which have been found to possess enzymatic activities. At least 20 different enzymes have been identified in pollen extracts (BLANCHARD & GARDNER, 1976).

Investigations of the natural origin chemical agent genotoxicity effects have provided us with strong reasons for engaging in a more comprehensive research of this heterogeneous group of compounds. Same natural products showed genotoxicity in experiments with *Drosophila melanogaster* (WILLIAMS *et al.*, 1980; MORGAN & HOFFMAN, 1983; SIMIĆ *et al.*, 2000). Therefore, the objective of the present paper was to investigate the genetic effects of the pollen acetone and watery extract of the plant *Ambrosia artemisiifolia* (L.) on *Drosophila melanogaster* using the SLRL test.

MATERIALS AND METHODS

Ambrosia artemisiifolia (L.) plants were collected from the region of Kragujevac, in central Serbia, and the biomass was freeze-dried in the same day. A voucher specimen of the plant was deposited in the Herbarium of the Department of Biology at the Faculty of Science, University of Belgrade, Serbia (BEOU No: 16171).

Pollen was broken into small pieces by using a cylindered crusher and pollen pieces (30g) were extracted with acetone using a Soxhlet apparatus and (30g) with water. The acetone extract of pollen was purified by thin layer chromatography on a MN-silica gel. The crude extract was analyzed with IC, H¹-NMR and MS.

The sex - linked recessive lethal test for mutagenicity (SLRL test) was performed with laboratory stocks of *Drosophila melanogaster* (obtained from the Umea Stock Centre, Sweden). *Canton-S*, line flies had a normal phenotype (*wild* type), while *Basc* line flies were characterized by individuals homozygous for an X-chromosome balancer carrying three genetic markers: *Bar* (*B*) which produces a narrow eye shape in homo- and hemizygous conditions and a kidney shaped eye when heterozygous in females. The character can be regarded as partially dominant; *white-apricot* (*w^a*) - alters the red eye colour into light orange and is expressed only in homozygous females and hemizygous males; *scute* (*sc*) - recessive mutation that reduces the number of thoracic bristles. This mutation is linked with the long inversion on the X-chromosome, necessary for suppression of crossover that could potentially change the existing gene combinations on the treated chromosome (LEE *et al.*, 1983).

Three-days-old *Canton-S* males (N=30+30; 30 for acetonic and 30 for watery extract) were starved in empty bottles for 5 h prior to the treatment, and then transferred and fed in bottles containing with filter paper soaked with a 5% solution of the acetone extract and in bottles with filter paper soaked with watery extract for 24 h. After another 24 h of recovery on a standard medium, each male was mated individually to three *Basc* females, in 30+30 bottles, which made brood I. After two days, males were transferred to new set of vials containing three virgins of *Basc* line (thus creating brood II). After three days, males were transferred again to the fresh vials with three *Basc* virgins (brood III). These males stayed with females for three days and were removed afterwards. Females were left alone for five days to lay eggs, and then they were removed. The solvent 1% *Sucrose* served as the negative control (LEWIS & BACHER, 1968).

After F₁ emerged, brother-sister mating was allowed for several days, and 10 females from each vial were placed individually into the new vials. Each vial would give the progeny of one treated X-chromosome. In F₂, the phenotypes were scored according to eye colour and shape. The absence of the *wild* type males indicated the presence of recessive lethal agent induced by the test substance.

The stocks were maintained, and all experiments were performed under the optimal conditions (t = 25°C, relative humidity = 60%, 12/12 hours light/dark regime) on a

standard nutritive medium for *Drosophila* (corn flour, yeast, agar, sugar and nipagin to prevent the occurrence of mould and infections).

The total number of treated X-chromosomes is equal to the sum of lethal and non-lethal cultures, and the frequency of sex-linked recessive lethal cultures was calculated according the ratio between the numbers of lethal cultures to the total number of treated X-chromosomes. A significance of the percentage difference regarding lethal cultures was performed through a testing for big independent samples (testing the difference between proportions - PETZ, 1985).

RESULTS AND DISCUSSION

Through examination of *A. artemisiifolia* (L.) many different kinds of metabolites including sesquiterpenic lactones, phenolics, coumarins and flavonoids have been identified (BLOSZYK *et al.*, 1992; TAMURA *et al.*, 2004; PARKHOMENKO *et al.*, 2006; MILOSAVLJEVIĆ *et al.*, 1999). Only two lactones, ambrosin and dihydroambrosin, were identified in the acetone extract (in the ratio of 3:1) of ambrosia pollen (Figure 1 and 2).

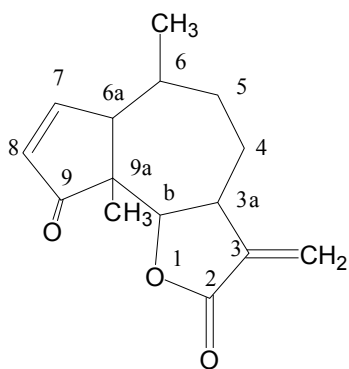


Figure 1. $C_{15}H_{18}O_3$, MW 246.30, 6,9a-dimethyl-3-methylene-3,3a,4,5,6,6a-hexahydroazuleno[4,5-b]furan-2,9(9aH,9bH)-dione (ambrosin)

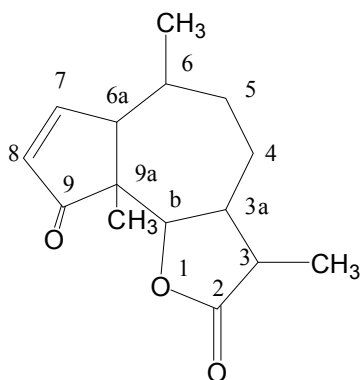


Figure 2. $C_{15}H_{20}O_3$, MW 248.30, 3,6,9a-dimethyl-3,3a,4,5,6,6a-hexahydroazuleno[4,5-b]furan-,9(9aH,9bH)-dione (dihydro 3-ambrosin)

α , β -Unsaturated lactones are the subject of interest in phytochemistry and medicine, due to their biological properties as cytotoxic, antitumor, antimicrobial agents and allergens (PARKHOMENKO *et al.*, 2005; KEBEDE, 1994) Previous literature gears us toward the fact that the existing pollen proteins are responsible for their allergenic activity (WAN *et al.*, 2002), although there is no proof whether those lactones actually contribute to such reaction along existing proteins as their carriers (MOLLER *et al.*, 2002; WARSHAW & ZUG, 1996).

Using short tests for the detection of mutagenicity in *Drosophila melanogaster* *in vivo* conditions and comparative analysis's acetonic and watery extract, we were able to determine a mutagenicity effect of the investigated plant (Table 1).

Table 1. Frequencies of SLRL mutations after treatment of *Drosophila melanogaster* males with acetonic and watery extracts of pollen of *Ambrosia artemisiifolia* (Statistically significant difference: $p < 0.01^{**}$; $p < 0.001^{***}$)

	Sucrose (Negative control)	Ambrosia (Acetonic extract)	Ambrosia (Watery extract)	t s/a (Acetonic extract)	t s/a (Watery extract)
I broods Σ	300	244	251	1,65	1,38
No Of lethal	5	10	9	$p > 0,05$	$p > 0,05$
% Of lethal	1,67	4,1	3,59		
II brood Σ	269	204	268	4,4	4,08
No of lethal	5	26	27	$p < 0,001^{***}$	$p < 0,001^{***}$
% Of lethal	1,86	12,74	10,04		
III broods Σ	252	236	194	2,97	2,68
No of lethal	6	20	16	$p < 0,01^{**}$	$p < 0,01^{**}$
% Of lethal	2,38	8,47	8,25		
I+II+III Σ	821	684	713	5,41	4,9
No of lethal	16	56	52	$p < 0,001^{***}$	$p < 0,001^{***}$
% Of lethal	1,95	8,19	7,29		

The frequency of germinative mutations induced by the pollen components is significantly higher than the frequency of mutations induced by sucrose (negative control). The obtained results showed that the spermatid cell line (brood II) is particularly sensitive to the extracts.

Since the statistically significant difference in the increase of frequency of the sex-related lethal in the tested group of males of *Drosophila melanogaster* (compared to a negative control) represents a positive result, it is concluded that a certain chemical component in the ambrosia pollen, induces mutations in male germinative cells of this eukaryotic species. Statistically significant differences in II and III brood, confirm the same sensitivity of the germinative cells of premeiotic (diploid) and postmeiotic stages (haploidic spermatid).

CONCLUSION

Results of comparative analysis acetonic and watery extracts showed that lactones, in acetonic, and same chemical substances in watery extract induces recessive, lethal X-linked mutations in postmeiotic germinative cell lines – spermatids and premeiotic line – spermatocytes, while the spermatozooids are more resistant to the genotoxicity effects of the investigated agents.

The experimentally proven genotoxicity of the ambrosia pollen extracts needs further investigation i.e., determination of the chemical structure of the pollen agent that is capable of inducing hereditary genetic changes in this *in vivo* system.

ACKNOWLEDGEMENTS

This study was financially supported by the Serbian Ministry of Science and Environmental Protection grants No 143008 and No 142025.

References:

- [1] BLANCHARD, G. C., GARDNER, R. (1976): Characterization of some of the enzymes in ragweed pollen. *Annals of Allergy* **36**: 410-418.
- [2] BLOSZYK, E., RYCHLEWSKA, U., CZEZAPANSKA, B., BUDESINSKY, M., DROZDZ, B., HOLUB, M. (1992): Sesquiterpene lactones of *Ambrosia artemisiifolia* L. and *Ambrosia trifida* (L.) species. *Collection Czechoslovak Chemical Communications* **57**: 1092-1102.
- [3] DAVID, J. P., de SANTOS, A. J. O., DA GUEDES, M. L. S., DAVID, J. M., CHAI, H. B., PEZZUTO, J.M., ANGERHOFERAND, C.K., CORDELL, A. (1999): Sesquiterpene lactones from *Ambrosia artemisiaefolia* (Asteraceae). *Pharmaceutical Biology* **37**: 165-168.
- [4] LEE, W. R., ABRAHAMSON, S., VALENCIA, R., VON HALLE, E. S., WURGLER, F. E., ZIMMERING, S. (1983): The sex-linked recessive lethal test for mutagenesis in *Drosophila melanogaster*: a report of the US Environmental Protection Agency Gene-Tox Program. *Mutation Research* **123**: 183-297.
- [5] LEWIS, E. B., BACHER, F. (1968): Method of feeding ethyl methane sulfonate (EMS) to *Drosophila* males. *Drosophila information service* **48**: 193.
- [6] KEBEDE, Z. (1994): *Allelopathic Chemicals*: Their Potential Uses for Weed Control in Agroecosystems.
- [7] MORGAN, R.W. & HOFFMAN, G. (1983): Cycasin and its mutagenic metabolites. *Mutation Research* **114**: 19-58.
- [8] MILOSAVLJEVIĆ, S., BULATOVIĆ, V., STEFANOVIĆ, M. (1999): Sesquiterpene lactones from Yugoslavia wild growing plant families *Asteraceae* and *Apiaceae*. *Journal Serbian Chemical Society* **64** (7, 8): 397-442
- [9] MOLLER, H., SPIREN, A., SVENSSON, A., GRUVBERGER, B., HINDSEN, M., BRUZE, M. (2002): Contact allergy to the Asteraceae plant *Ambrosia artemisiifolia* L (ragweed) in sesquiterpene lactone-sensitive patients in southern Sweden. *Contact Dermatitis* **47** (3): 157-160.
- [10] PARKHOMENKO, A. Y. U., ANDEEVA, O. A., OGANESYAN, E. T., IVASHEV, M. N. (2005): *Ambrosia artemisiifolia* as a source of biologically active substances. *Pharmaceutical Chemistry Journal* **5**: 149-153.

- [11] PARKHOMENKO, A. Y. U., OGANESYAN, E. T., ANDREEVA, O. A., DORKINA, E. G., PAUKOVA, E. O., AGADZHANYAN, Z. S. (2006): Pharmacologically active substances from *Ambrosia artemisiifolia*. Part 2. *Pharmaceutical Chemistry Journal* **40**: 627-632.
- [12] PETZ, B. (1985): *Osnovne statističke metode za nematematičare*. SNL Zagreb. Croatia.
- [13] RUGUTT, K. J., FRONCZEK, F. R., FRANZBLAU, S. G., WARNER, I. M. (2001): Dihydroparthenolide diol, a novel sesquiterpene lactone. *Acta Crystallographica E* **57**: 0323-0325.
- [14] SIMIĆ, D., KNEŽEVIĆ-VUKČEVIĆ, J., VUKOVIĆ-GAČIĆ, B. (2000): Prospects in using medicinal and aromatic plants in cancer prevention. *Proceedings of the First Conference on Medicinal and Aromatic Plants Southeast European Countries*, 97-104.
- [15] TAMURA, Y., HATORI, M., KONNO, K., HONDA, H., ONO, H., YOSHIDA, M. (2004): Triterpenoid and caffeic acid derivatives in the leaves of ragweed, *Ambrosia artemisiifolia* L. (Asterales: Asteraceae), as feeding stimulants of *Ophraella communa* LeSage (Coleoptera: Chrysomelidae). *Chemoecology* **14**: 113-120.
- [16] TAO, A. L., HE, S. H. (2005): Cloning, expression and characterization of pollen allergens from *Humulus scandens* (Lour) Merr and *Ambrosia artemisiifolia* L. *Acta Pharmacologica Sinica* **26** (10): 1225-1232.
- [17] WILLIAMS, G. M., MORI, H., HIRONO, I., NAGAO, M. (1980): Genotoxicity of pyrrolizidine alkaloids in the hepatocyte primary culture DNA-repair test. *Mutation Research* **79**: 1-5.
- [18] WANG, P., KOMG, C. H. H., ZHANG, C. H. X. (2006): Chemical composition and antimicrobial activity of the essential oil from *Ambrosia trifida* L. *Molecules* **11**: 549-555.
- [19] WAN, S., YUAN, T., BOWDIISH, S., WALLACE, L., RUSSELLI, S. D., LUO, Y. (2002): Response of an allergenic species, *Ambrosia psilostachya* (Asteraceae), to experimental warming and clipping: implications for public health, *American Journal Botany* **89**: 1843.
- [20] WARSHAW, E. M., ZUG, K. A. (1996): Sesquiterpene lactone allergy, *American Journal Contact Dermatitis* **7** (1): 1-23.