MAKÜ FEBED
ISSN Online: 1309-2243
http://febed.mehmetakif.edu.tr

Mehmet Akif Ersoy Üniversitesi Fen Bilimleri Enstitüsü Dergisi 3(2): 7-11 (2012)

Research Paper / Araştırma Makalesi

Developmental disorders caused by *Verbascum speciosum* Schrad. Extracts in *Drosophila melanogaster* (Diptera: Drosophilidae)

Handan Uysal[a], Özkan Aksakal[a], Hakan Aşkın[b]

[a]Department of Biology, Science Faculty, Atatürk University, 25240 Erzurum, Turkey
[b]Department of Molecular Biology and Genetics, Science Faculty, Atatürk University, 25240 Erzurum, Turkey

Received (Geliş Tarihi): 29.12.2011, Accepted (Kabul Tarihi): 05.16.2012
Corresponding author (Yazışmalardan Sorumlu Yazar): hauysal@atauni.edu.tr (H. Uysal)
0 442 231 43 36 0 442 236 09 48

ABSTRACT

The effect of aqueous seed extracts of *Verbascum speciosum* on the development biology of *Drosophila melanogaster* was determined. Various doses of boiled and unboiled extracts (2, 4, 6, 8 and 10 mg/100 mL standard Drosophila medium (SDM)) were added to the standard medium of *D. melanogaster*. The use of the seed extracts significantly increased the mortality of the F_1 generation. Emerged individuals belonging to F_1 decreased with an increase in dose ($P<0.05$). In addition, severe malformations were observed belonging to different extremities in the newly-emerged flies of F_1 progeny. The results showed that unboiled seed extracts of *V. speciosum* are more toxic to *D. melanogaster* than their boiled counterpart (Vsb<Vsub).

Key Words: *Drosophila melanogaster*, *Verbascum speciosum*, developmental biology, mortality

Verbascum speciosum Schrad. Ekstraktının *Drosophila melanogaster*'de Neden Olduğu Gelişim Bozuklukları

ÖZET

Bu çalışmada, *Drosophila melanogaster*'in gelişim biyolojisi üzerine *Verbascum speciosum*'a ait tohumların su ekstraktlarının etkileri araştırılmıştır. Bu amaçla, kaynatılmış ve kaynatılmamış tohum ekstraktlarının farklı dozları (2, 4, 6, 8 ve 10 mg/100 mL SDB) *D. melanogater*'in standart besiyerine ilave edilmiştir. Bu ekstraktların kullanılmasıyla F_1 neslinde ölüm oranının önemli bir ölçüde arttığı gözlenmiştir. Doz artışına bağlı olarak F_1 nesline ait birey sayısı azalmıştır ($P<0.05$). Ayrıca F_1 nesline ait yavru bireylerin çeşitli ekstremitelerinde ciddi malformasyonlar gözlenmiştir. Çalışma sonucunda, *V. speciosum*'a ait kaynatılmamış ekstraktın kaynatılmış ekstrakta göre daha toksik olduğu gözlenmiştir (Vsb<Vsub).

Anahtar Kelimeler: *Verbascum speciosum*, *Drosophila melanogaster*, gelişim biyolojisi, mortalite

1. INTRODUCTION

Verbascum L. is a member of the Scrophulariaceae family. This genus is represented by 228 species, 185 of which are endemic to Turkey (Huber-Morath, 1978). *Verbascum* species contain a wide range of compounds such as alkaloid (Youhnovski et al., 1999), glycosides (Klimek, 1996; Skaltsounis et al., 1996; Elgindi & Mabry,

2000) and saponins (Hartleb & Seifert, 1994). Therefore, some species of *Verbascum* have been commonly used to treat internal and external infections for centuries. Many studies have so far demonstrated that these species exhibit various biological activities and can be used to treat rheumatic pain, superficial fungal infections, pulmonary complaints, eczema and other types of inflamed skin conditions (Baytop, 1999).

The leaves and flowers of *Verbascum* are reported to have expectorant, mucolytic and demulcent properties which are used to treat respiratory problems such as tuberculosis, asthma, and dry coughs in traditional Turkish medicine (Baytop, 1999). *Verbascum* flowers are boiled in milk and reapplied externally for pruritic conditions affecting the urogenital organs. Iridoid glycosides are widely found in the *Verbascum* genus. Studies on the *Verbascum* species have particularly reported on aucubin, catalpol, and their acyl derivatives (Tatli et al., 2003; Tatli et al., 2004; Akdemir et al., 2005). Iridoids have been shown to possess a number of biological activities including antidiabetic, anti-inflammatory, anticancer and immunostimulant activities (Vijayavitthal et al., 1998; Konoshima et al., 2000; Stevenson et al., 2002; Ahmed et al., 2003). Furthermore, the presence of iridoid glycosides has been suggested to have a taxonomic importance.

In particular, the flowers and leaves of *Verbascum phlomoides* and *Verbascum thapus* species have been used for their ethnopharmacological effects by Turkish people (Dulger et al., 2007). The seeds of *Verbascum* species are poisonous and thus should not be used unlike the other parts of plant. The seeds are claimed to intoxicate fish when thrown into water and therefore used by poachers for their slightly narcotic effects. They include major toxic elements that affect the circulatory, respiratory and central nervous systems of the fish since they contain agents such as saponin, rotenone and glycoside (Turker & Gurel, 2005).

Verbascum speciosum is a biennial plant of eastern Anatolia that grows mostly in dry and rocky places and there are no reports on its detailed studies. Thus, we were interested in investigating the plant more thoroughly. The using of bioactive compounds of plants ranges from pharmaceutical industry to the agricultural pest control. In the last decades, researchers seeking plant extracts bioactivity; it is necessary using a model organism that this plays how the compounds reveal this bioactivity. Model organism can be used in investigation research concerning plant extracts bioactivity. *Drosophila melanogaster* is considered one of the best models, because is easy to handle, requires small space and allows large number of repetitions (Graf et al., 1984). *Drosophila melanogaster* has been an organism used for bioassays to evaluate toxic and genotoxic effects of various plants. There is no adequate investigation on the toxic effects of the seed-water extract of *V. speciosum.* On the other hand, there is no study on the effects of seed-water extract on *D. melanogaster*. In this study, we aimed to investigate the toxic effects of the seed extract of *V. speciosum* on the offspring production and developmental time of *Drosophila melanogaster*.

2. MATERIAL and METHODS

2.1. Collection and preparation of the water extract of *Verbascum speciosum* seeds

Verbascum speciosum seeds were collected from the vicinity of Erzurum/Turkey province. Approximately, 20 g seeds of this plant were obtained and homogenized for 10 min in 100 mL distilled water by using a Warring blender. Fifty percent of the extracts were boiled for 1 h and the rest was kept unboiled. Both types of extracts were filtered through a filter paper (Whatman no. 1) and kept in a freezer at -20°C.

2.2. Experimental animal and application of the extract of *Verbascum* seeds to the experiment group

The flies used in the experiments were Oregon R wild type (w.t.) strain of *Drosophila melanogaster* (Diptera; Drosophilidae). This stock had been maintained for many years in the Laboratory at the Department of Biology of the Atatürk University in Erzurum and was, therefore, highly inbred with little genetic variation. Because of the mutagenic properties can easily be observed in *Drosophila melanogaster*, this organism has been often used in genetic experiments. The flies were kept at a constant temperature of 25 ± 1°C on standard medium composed of maize-flour, agar, sucrose, dried yeast and propionic acid (Standard Drosophila Medium = SDM). The flies were kept in darkness, except during the transfers onto fresh medium (usually twice weekly). The humidity of the experimental chamber was 40-60%. The females used in this experiment were virgins.

Five different concentrations of both boiled (Vsb) and unboiled homogenates of *Verbascum speciosum* (Vsub) extracts (2, 4, 6, 8, 10 mg/100 mL SDM) were added to 100 mL of SDM and kept at room temperature to diffuse the extract on the medium. Flies of the same age were used for experimental purposes. Virgin females and males were collected from synchronized cultures. The culture vials containing only SDM were used as the control group. The treatment and control vials were kept at 25±1°C and a relative humidity of 60%. To characterize the time of development, ten female and ten male flies were mated. After emerging, the developmental time was followed daily. Beginning from the first adult emergence, the number and sex of the living adult flies emerging from each vial were recorded daily. The experiments were repeated three times for each group.

To determine difference between control and application groups, data were subjected to Duncan's Multiple Range Test using SPSS 11.5 software.

3. RESULTS

In the laboratory conditions, at 25±1°C, the life cycle of *D. melanogaster* was found to be nine days. In the control and all experimental groups (Vsb and Vsub), laid

eggs were observed on the second day of mating. The first obtained individuals of the F_1 generation for control and Vsb experimental groups were observed on the 9th day. However, developmental stages were delayed for all doses of Vsub (larvae-to-adult). Particularly, for 6.0, 8.0 and 10.0 mg/mL application groups, the first adult was formed on the 11th, 15th and 13th days, respectively.

As demonstrated in Table 1, the total F_1 progeny significantly decreased with increased doses of Vsb and Vsub. While the total number of F_1 progeny was 2618 for the control group, the figure ranged from 1944 to 1266 for Vsb and from 638 to 746 for Vsub (Fig. 1). The differences between the treatment and control groups were significant ($P<0.05$).

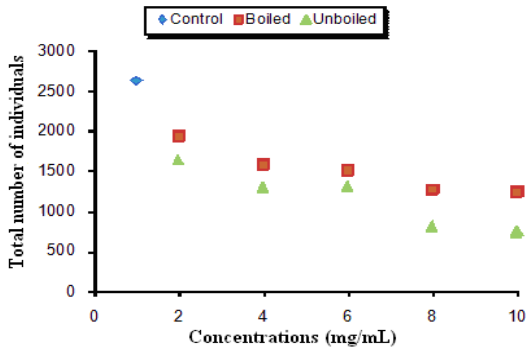

Figure 1. Comparisons of the toxic effects of boiled and unboiled seed extracts on the total number of *D. melanogaster* offsprings

In our experiments, we observed severe malformations in different organs of the offspring flies of F_1 progeny, such as shortened legs due to fusion of tarsal segments, unopened wings, and lack of thorax formation. Malformation rates for all groups are given in Table 1. While the rate of malformed individuals was 0.38% in the control group, this rate ranged from 0.56% to 1.66% in Vsb and from 0.73% to 4.83% in Vsub. As seen in Table 1, Vsub is usually more toxic than Vsb and a positive correlation was found between the dose and the number of malformed individuals.

Table 1. The effects of boiled and unboiled seed extracts of *V. speciosum* on the number of the offsprings of *D.melanogaster*

Boiled (mg/10mL SDM)	♂♂	♀♀	Σ	Σ Malf. (%)	Unboiled (mg/100mL SDM)	♂♂	♀♀	Σ	Σ Malf. (%)
Control	1301	1317	2618[a]	10 (0.38)	Control	1301	1317	2618[a]	10 (0.38)
2.0	903	1041	1944[b]	11 (0.56)	2.0	825	813	1638[g]	12 (0.73)
4.0	765	813	1578[c]	15 (0.95)	4.0	621	672	1293[h]	18 (1.39)
6.0	738	777	1515[d]	19 (1,25)	6.0	567	738	1305[h]	27 (2.07)
8.0	576	690	1266[e]	21 (1.66)	8.0	378	432	810[k]	33 (4.07)
10.0	684	654	1338[f]	18 (1.35)	10.0	339	407	746[l]	36 (4.83)

Differences between the experiment and control groups are significant at the level of 5% according to Duncan's Multiple Range Test for F_1 generations ∑: (♂♂+♀♀) the total number offsprings (Malf %): Percentage malformed offsprings

The results of the current study suggest that this plant extract has strong effects on the development of *D. melanogaster*. Its effect is dose-dependent. The life cycle of the offsprings of the extract-applied parents was longer than the control group (approximately between 9 and 13 days). F_1 progeny production of *D. melanogaster* was inhibited by unboiled and boiled extracts of *V. speciosum*.

4. DISCUSSION

Many plant essential oil, extracts and phytochemicals are known to possess toxic activity against *Drosophila melanogaster*. Naturally occuring toxic phytochemicals are isothiocyanates and glucosinolates from Brassicaceae, cyanogenic glicosides from Asteraceae, Saponins and alkaloids from Scrophulariaceae. Since, to the best of our knowledge, there are a few studies in the literature on the biological activity of *Verbascum speciosum*, the results of our study will be evaluated in the light of some studies reported previously. The results of the present study demonstrated that experimental dosage of aqueous extract of *Verbascum speciosum* influenced negatively both offspring production and developmental time. From the obtained results, we have drawn several conclusions. Firstly, most plant extracts have insecticidal properties and can control pests by affecting other biological activities (Schmutterer, 1995; Mostafa et al., 1996). Secondly, treatment with such plant extracts inhibited oviposition and progeny emergence (Khoshnoud & Khayamy, 2008).

As like our study Khoshnoud et al., (2008) studied toxic effect of *Verbascum speciosum* against *Sitophilus*

oryzae species and they found that the mortality of adults increased with the increase of dose. Similar observations have been made about the effects of other plant extracts. For example, Sadek, (2003) observed that the time of pupation in the larvae of *Spodoptera littoralis* (Boisduval) was increased by the extract of *Adhatoda vasica.* Jeyabalan et al., (2003) have reported that extract of *Plargonium citrosa* (Vanleenii) prolonged the duration of larval instars and total developmental time of *Anopheles stephensi.* According to Zhong et al., (2001) extract of *Rhododendron molle* flowers extend the developmental duration of *Pieris rapae.* Similarly, the extract of *V. cheiranthifolium* both suppressed oviposition and killed the- larvae hatching from the eggs of *Sitophilus oryzae* and *Tribolium castaneum* (Herbst.) (Khoshnoud et al., 2008). Larval and pupal toxic effects caused by *Euphorbia canariensis* have also been demonstrated by various authors in *Musca domestica* (Beard & Walton, 1971) and *D. melanogaster* (Uysal & Kaya, 2004).

Giner et al., (2000) reported that verbascosaponin was obtained from seeds of *Verbascum* species. Saponins caused poisoning in different organisms (Turker & Gurel, 2005). Extracts obtained from seeds of these species demonstrated a strong inhibitory effect on the elongation step of protein biosynthesis (Paszkiewicz-Gadek et al., 1990; Kupeli et al., 2007). In addition, these saponins were also shown to have significant cytotoxic activities in rat liver (Tatli et al., 2004). It is well possible that developmental irregularities observed in *D. melanogaster* were caused by similar effects. Consequently, this study suggests that water extract of the seeds of *V. speciosum* possesses toxic features with significant insecticidal effects and could be potentially used as safe and cheap insecticides.

5. REFERENCES

Ahmed, B., Al-Rehaily, A.J., Al-Howiriny, T.A., El-Sayed, K.A., Ahmad, M.S. (2003). Scropolioside-D2 and harpagoside -B: two new iridoid glycosides from *Scrophularia deserti* and their antidiabetic and anti-inflammatory activity. *Biol Pharm Bull,* 26, 462–467.

Akdemir, Z.S., Tatli, I.I., Bedir, E., Khan, I.A. (2005). Two new iridoid glycosides from *Verbascum salviifolium,* Boiss. *Zeitschrift fur Naturforschung B,* 60, 113–117.

Baytop, T. (1999). Therapy with Medicinal Plants in Turkey (Past and Present), Nobel Tıp Kitabevleri Ltd., İstanbul, pp. 334.

Beard, R.L, Walton, G.S. (1971). Insecticidal mycotoxins produced by *Aspergillus flavus* var. colunaris. *Bull Conn Agr Exp Sta,* 725, 1-26.

Dulger, B., Kirmizi, S., Arslan, H., Güleryüz, G. (2007). Antimicrobial activity of three endemic *Verbascum* species. *Pharm Biol,* 40, 587–589.

Elgindi, M.R., Mabry, T.J. (2000). Phenyletanoid glycosides from *Verbascum siniaticum*. *Asian J Chem,* 12, 127–130.

Giner, R.M., Villalba, M.L., Recio, M.C., Manez, S., Cerda-Nicolas, M., Rios, J.L. (2000). Anti-inflammatory glycoterpenoids from *Scrophularia auriculata*. *Eur J Pharm,* 389, 243–252.

Graf, U., Würgler, F.E., Katz, A.J., Frei, H., Juon, H., Hall, C.B., Kale, P.G. (1984). Somatic mutation and recombination test in *Drosophila melanogaster. Environ Mutagen,* 6 (2), 153-188.

Hartleb, I., Seifert, K. (1994). Sangarosaponin-D a triterpenoid saponin from *Verbacsum songaricu. Phytochem,* 35, 1009–1011.

Huber-Morath, A. (1978). *Verbascum* in Flora of Turkey and The East Aegean Islands, Vol. 6, Ed: Davis, P.H., University Press, Edinburgh, pp. 461-603.

Jeyabalan, D., Arul, N., Thangamathi, P. (2003). Studies on effects of *Plargonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* Liston. *Bioresour Technol,* 89, 185-189.

Khoshnoud, H., Khayamy, M. (2008). Insecticidal effects of ethanolic extract from *Verbascum cheiranthifolium* Boiss. against two stored-product insect pests species. *J Biol Sci,* 8(1), 191-195.

Khoshnoud, H., Ghiyasi, M., Amirnia, R., Fard, SS. (2008). The potential of using insecticidal properties of medicinal plants against insect pest. *Pak J Biol Sci,* 11(10), 1380-1384.

Klimek, B. (1996). Hidroxycinnamoyl ester glycosides and saponins from flowers of *Verbascum phlomoides. Phytochem***,** 43, 1281–1284.

Konoshima, T., Takasaki, M., Tokuda, H., Nishimo, H. (2000). Cancer chemopreventive activity of an iridoid glycoside, 8-acetylharpagide from *Ajuga decumbens*. *Cancer Lett,* 157, 87–92.

Kupeli, E., Tatli, I.I., Akdemir, Z.S., Yesilada, E. (2007). Bioassay-guided isolation of anti-inflammatory and antinociceptive glycoterpenoids from the flowers of *Verbascum lasianthum* Boiss. ex Bentham. *J Ethnopharmacol,* 110, 444-450.

Mostafa, T.Y., Mahgoub, S.M., Ahmed, M.S. (1996). The efficiency of certain plant powders against cowpea weevil *Callosobruchus maculates* (F.) (Coleoptera: Bruchidae). *Egypt J Agric Res,* 74, 307-319.

Paszkiewicz-Gadek, A., Grochowska, K., Galasinski, W. (1990). Effect of the aqueous extract and saponin fraction from the flowers of *Verbascum thapsiforme* on protein biosynthesis in a rat liver ribosomal system. *Phytother Res,* 4 (5), 177–181.

Sadek, M.M. (2003). Antifeedant and toxicity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lep., Noctuidae). *J. Applied Entomol,* 127, 396-404.

Schmutterer, H. (1995). The neem tree *Azadirachta indica* A. juss and other malicious plant sources of unique natural products of integrated pest management medicine, industry and other purposes,VCH, Veinheim, Federal Republic of Germany.

Skaltsounis, A.L., Tsitsa-Tzardis, E., Demetzos, C., Harvala, C. (1996). Unduloside, a new iridoid glycoside from *Verbascum undulatum. J Nat Prod,* 59, 673–675.

Stevenson, P.C., Simmonds, M.S.J., Sampson, J., Houghton, P.J., Grice, P. (2002). Wound healing activity of acylated iridoid glycosides from *Scrophularia nodosa*. *Phytother Res,* 16, 33–35.

Tatli, I.I., Akdemir, Z.S., Bedir, E. (2003). 6-*O*-l-Rhamnopyranosylcatalpol derivative iridoids from *Verbascum cilicicum*. *Turk J Chem,* 27, 765–772.

Tatli, I.I., Akdemir, Z.S. Bedir, E. (2004). Saponin, iridoid, phenylethanoid and monoterpene glycosides from *Verbascum pterocalycinum* var. mutense. *Turk J Chem,* 28, 111–122.

Turker, A.U., Gurel, E. (2005). Common Mullein (*Verbascum thapsus* L.) Recent Advances in Research, *Phytother Res,* 19, 7-13.

Uysal, H., Kaya, Y. (2004). Toxicity of *Euphorbia canariensis* latex to some developmental stages of *Drosophila melanogaster* Diptera: Drosophilidae). *Bull Environ Contam Toxicol,* 72, 45-53.

Vijayavitthal, T.M., Kanwal, R., Amiya, P.B., Ragini, S., Anju, P., Lalit, M.T., Vishwa, M.L.S. (1998). Studies on the profile of immunostimulant activities of modified iridoid glycosides. *Bioorg Med Chem,* 6, 605–611.

Youhnovski, N., Dandarov, K., Guggisberg, A., Hesse, M. (1999). Macrocyclic spermine alcholoids from *Verbascum* Isolation, structure elucidation and synthesis of the (*E*/*Z*)-isomeric pairs(*S*)-verbasikrine/(*S*)- isoverbasikrine and (*S*)-verbamekrine/(*S*)-isoverbamekrine, *Helv Chim Acta,* 82, 1185–1194.

Zhong, G.H., Hu, M.Y., Weng, A.Q. (2001). Laboratory and field evaluations of extracts from *Rhododendron molle* flowers as insect growth regulator to imported cabbage worm, *Pieris rapae* L. (Lepidoptera:Pieridae). *J Appl Entomol,* 125, 563-596.