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Recovering Effects of Aqueous Extracts of Some Selected Medical Plants on the Teratogenic Effects During the Development of *D. melanogaster*

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Abstract: In this study the effects of some selected medical plants (*Pimpinella anisum* L., *Rosmarinus officinalis* L., *Achillea millefolium* L., *Acorus calamus* L., *Hypericum perforatum* L.) on the development of *Drosophila melanogaster* have been investigated. When the different concentration of plant extracts were applied to the cultures of *Drosophila melanogaster*, they did not caused an elongation of metamorphosis of F₁ progeny. Furthermore, depending on an increase of plant extract on the application groups, the number of offsprings increased. But this increasing (for application groups no. I, II and IV) was not statistically significant ($p > 0.05$) according to control group. The highest increase in the total number of offspring of F₁ progeny obtained from applications of *Acorus calamus* extracts and the 10 mL/100 mL medium concentration of the extract of *Hypericum perforatum*.

Key words: *D. melanogaster*, medical plants, recovery effects, reproduction, development

INTRODUCTION

Recent years, many investigations have been done searching new substances from various sources, like medicinal plants, which are the good sources of therapeutic agents. (Shukla *et al.*, 2002; Schempp *et al.*, 2005; Menegazzi *et al.*, 2006). For this purpose, both in medical research and in biological research, more attention is paid to the antioxidant properties of medicinal plants to minimize the harmful effects of radicals. For example, according to Menegazzi *et al.* (2006). *H. perforatum* extract reduces the development of acute inflammation in mice. *Acorus calamus* extract also showed protective effect in the rat brain induced by noise-stress (Manikandan *et al.*, 2005). Exposure of rats to acrylamide caused hind limb paralysis. But in rats neurobehavioral changes produced by acrylamide prevented following treatment with *A. calamus* rhizomes (Shukla *et al.*, 2002). Again, extracts of rosemary, *Rosmarinus officinalis*, have been used in wistar rat at the organogenic period of pregnancy and no observed anomalies/malformations in the term fetuses (Lemonica *et al.*, 1996). Besides, maternal toxicity were also not observed in rats at exposed *H. perforatum* (Borges *et al.*, 2001).

In Turkish folk medicine, many traditional medicinal plant species which have been used for various purposes, such as sedatives, tranquilizers, diuretics and expectorants and for diaphoretic activities (Acartürk, 1997; Baytop, 1999; Eröztürk, 2001). From this

plants, *Pimpinella anisum*, *Rosmarinus officinalis*, *Achillea millefolium*, *Acorus calamus*, *Hypericum perforatum* are especially used in folk medicine for their recovering effect on digestive system disorder (Kuhn and Winston, 2001). Furthermore, it has been reported that these five plants also have antioxidant activity (Kara, 2002). It is well known that Reactive Oxygen Species (ROS) have been implicated in more than 100 diseases, including malaria, acquired immune deficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes and cancer (Tanizawa *et al.*, 1992; Alho and Leinonen, 1999). Nevertheless, all aerobic organisms, including human beings, have antioxidant defences that protect against oxidative damages. However, this nature antioxidant mechanism can be inefficient, hence, dietary intake of antioxidant compounds becomes important (Halliwell, 1994; Terao *et al.*, 1994).

Therefore, research for the determination of source of the natural antioxidants is important. The various activity of plant extracts, like antimicrobial, antioxidant, toxic and mutagenic, have been widely reported (Yeşilada *et al.*, 1993; Perich *et al.*, 1994; Valsaraj *et al.*, 1997; Ali-Shtayeh *et al.*, 1998; Sakthivadivel and Thilagavothy, 2003). If a plant extract is used to treat a disease, it is prefer to have high antioxidant activity and have not a toxic and mutagenic effect on used organism.

Unfortunately, there is no enough literature about the effect of medicinal plants on *Drosophila melanogaster* (Diptera). We aimed to test the teratogenic effects of some medical plants with antioxidant activity (Kara, 2002) on the development of *D. melanogaster*.

MATERIALS AND METHODS

Plant materials: This study has been realized at Erzurum, Turkey, between 2005-2006 years. Five plant species, *Pimpinella anisum* L., *Rosmarinus officinalis* L., *Achillea millefolium* L., *Acorus calamus* L., *Hypericum perforatum* L., were obtained as dried plants from a market (Arifoglu mark). Scientific and local names, parts used and folk uses of these plants were summarized in Table 1.

Preparation of plant extracts: To prepare extracts, 10 g of powdered parts of plants (used parts, Table 1) were added to 250 mL flasks containing 100 mL water. The mixtures were incubated at room temperature in a rotary shaker (250 revolution per minute) for 3 days. Final suspension was sterilized by filtration with membrane filter (0.2 µm). Filtrate were stored at refrigerator until used (Srinivasan *et al.*, 2001)

Organisms: Oregon-R strain of *Drosophila melanogaster* was used for present studies. Because of the mutagenic

properties can easily be observed in *D. melanogaster*, this organism has been oftenly used in genetic experiments. The flies were reared on a standard food medium containing of commmeal-yeast-agar-sugar and added propionic acid as antimouldant (Standard *Drosophila* Medium = SDM). The three different concentrations (1.0, 5.0 and 10 mL/100 mL medium) of plant extracts (PE_s) was mixed into 100 mL of SDM and kept at room temperature waiting for 24 h to diffusion of PEs to the medium. The culture vials containing only the SDM were used as control. To study the effect of PE_s on development, virgin parents (especially females) with the same age were mated. Eggs belong to F₁ were allowed to develop in the different concentrations of PEs and control at uniform temperature 25±1°C. After emergence the flies were counted and examined phenotypic properties under the binocular microscope every day. This procedure was repeated three times for both control and applied groups (Uysal and Kaya, 2004).

Data analysis: Statistical analysis of data was done using Duncan's one-way range test and SPSS 11.5 for Microsoft Windows.

RESULTS AND DISCUSSION

Different concentrations (1.0, 5.0 and 10 mL/100 mL medium) of five PE_s were applied to the adult individuals of *D. melanogaster*. A repeat control group (mean of

Table 1: Used medical plants and their some characteristics

Species	Local name	Parts used	Folk uses*
<i>Pimpinella anisum</i> L. (Umbelliferae)	Anason	Fruits	Infections, angina, bronchitis, gastritis, laryngitis, migraine
<i>Rosmarinus officinalis</i> L. (Labiatae)	Biberiye	Leaves	Antiseptic, jaundice, migraine, stomach ache, asthma, edema
<i>Achillea millefolium</i> L. (Compositae)	Civanperçemi	Flowers and leaves	Infections, itch, skin injuries, stomach ache, menopause, ulcer, hemorrhoid
<i>Acorus calamus</i> L. (Araceae)	Eğir kökü	Rhizomes	Dysentery, jaundice, rachitism stomach ache, digestive problems, cirrhosis,
<i>Hypericum perforatum</i> L. (Guttiferae)	Kantaron	Flowers	Bronchitis, skin injuries, stomach ache, rheumatism, diarrhea, hemorrhoid, asthma antidepressant, tuberculosis

*References for used medical plants (Acarturk, 1997; Baytop, 1999)

Table 2: The total number of offsprings and malformed individuals in the progeny F₁ of parents treated with PE.

Control	Application groups	Concentrations (mL/100mL medium)		
		1.0 Σ (Malf %)	5.0 Σ (Malf %)	10 Σ (Malf %)
2870 (6-%0.2)	<i>P. anisum</i> (I)	2850 ^{ns} (7-0.2)	2872 ^{ns} (3-0.1)	2912 ^{ns}
	<i>R. officinalis</i> (II)	2791 ^{ns} (9-0.3)	2856 ^{ns} (4-0.1)	2936 ^{ns}
	<i>A. calamus</i> (III)	3248* (5-0.1)	3200*	3384*
	<i>H. perforatum</i> (IV)	2852 ^{ns} (2-0.07)	2916 ^{ns}	3224*
	<i>A. millefolium</i> (V)	2735 ^{ns} (3-0.1)	2751 ^{ns}	2803 ^{ns}

Malf : Rate of malformed individuals, Σ: Total number of offsprings (male+female), ns: non-significant vs. control (p>0.05), *: vs. control (p<0.01)

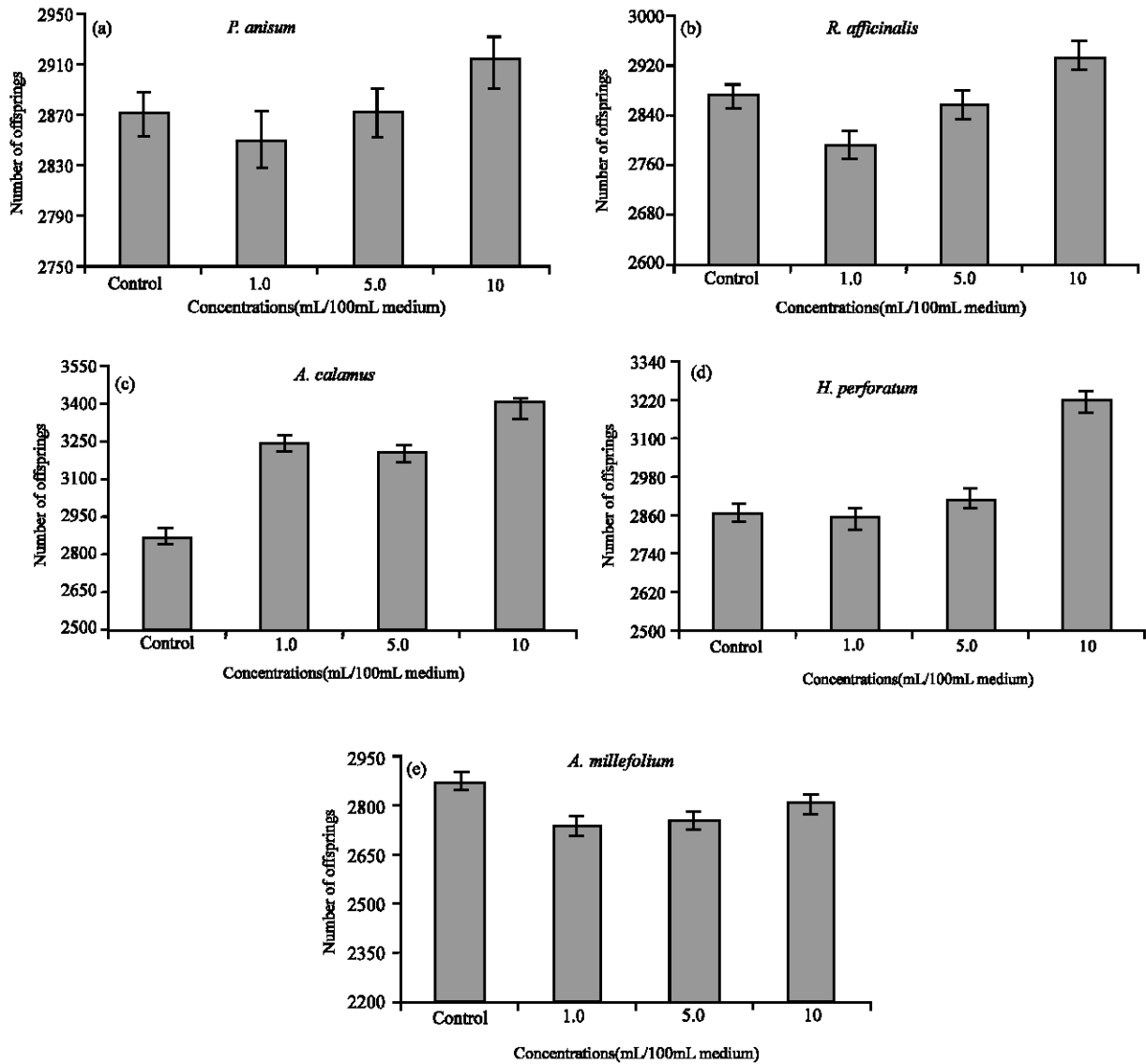


Fig. 1: The comparison of effects on the total number of offsprings of *P. anisum*, *R. officinalis*, *A. calamus*, *H. perforatum* and *A. millefolium* extracts at different concentrations

three independent experiments) was used for each PE_s groups. Firstly, developmental time was followed from day of egg deposition to day the adult eclosed. Eclosion of flies in control and PE_s (for all of the applied groups) started on 9th day and finished on 17th day. These results indicated that PE_s did not caused an elongation of metamorphosis. Results of F₁ progeny were summarized at Table 2. As seen in Table 2, the number of the offsprings were increased with increasing concentrations of PE_s. But, when this differences were compared with their control group, the effects of the extracts of *P. anisum* (application group No.I, Fig. 1a), *R. officinalis* (application group No.II, Fig. 1b) and *A. millefolium* (application group

No.V, Fig. 1e) were not statistically important ($p > 0.05$). However, at the application of *A. calamus* (application group No.III, Fig. 1c) 3248, 3200, 3384 individuals were counted from the lower concentration to higher concentrations and these differences were found to be significant ($p < 0.01$). Similar results were also observed in the application of *H. perforatum* (application group No.IV, Fig. 1d). Especially, the increasing of number of offsprings at highest concentration (10 mL/100 mL medium) were significant ($p < 0.01$). The ranking of recovery effect of the five extracts was *A. Calamus* > *H. perforatum* > *R. officinalis* > *P. anisum* \geq *A. millefolium*.

On the other hand, some malformations (especially in wing, thorax and legs) were observed in both control (0.2%) and application groups (0.07-0.3%). The number of these malformations were also given for each PE_s groups in Table 2. As can be seen in Table 2, the numbers of malformed individuals were 2-9 at lowest concentrations (1.0 mL/100 mL medium) and 6 for control group. Surprisingly, at the application of *P. anisum* and *R. officinalis* the number of malformed individuals were decreased with increasing concentrations of PE_s and there was no malformed individuals at the highest concentrations (10 mL/100 mL medium). Again, any malformed individuals were observed at the 5 and 10 mL/100 mL concentrations of PE_s of *A. calamus*, *H. perforatum* and *A. millefolium*.

According to above results, it can be said that none of PE_s has toxic and mutagenic effect on *D. melanogaster*. Because, the number of offsprings were increased with the increasing PE_s. In this case, we said that the PE_s have not adverse effect on the fecundity and viability of *D. melanogaster*. Even, they decreased the number of malformed individuals.

According to Kara (2002), these five plants have antioxidant properties. The plants that rich in antioxidant materials are stated to have recovery properties with active ingredients. Major active ingredients of these plants are achilleine (=betonicine) for *A. millefolium* (Wren, 1988), anethol (anethole-glycol) for *P. anisum* (Pourgholam *et al.*, 1999, Boskabady and Ramazani-Assari, 2001), rosmarinic acid (=rosmarinic) for *R. officinalis* (Ames *et al.*, 1993), β -asarone for *A. calamus* (Vohora *et al.*, 1990; Shukla *et al.*, 2002) and hypericin (=hyperiforin) for *H. perforatum* (Wagner and Bladt, 1994). Similar effects have been reported on some other organisms. For example, neither teratogenic effect nor malformations were observed at the fetuses of wistar rat pregnancy feed with extracts of rosemary, *Rosmarinus officinalis* L. (Lemonica *et al.*, 1996). Again, according to Sotelo-Felix *et al.* (2002), rosmarinus is antioxidant and prevents acute liver damage in rats. Previous research observed antimutagenic effects of rosemary phenolics against chromosomal damage induced in human lymphocytes by gamma ray, too (Del Bano *et al.*, 2006). Recovery effects of *P. anisum* was also shown against clastogenicity induced-arsenite in mice (Odonola, 2003). Hypericin, *Hypericum perforatum* L., were produced a well therapeutic response in mice inoculated with fibrosarcoma cells and regressed rate of tumours (Cavarga *et al.*, 2005). Recent studies also have indicated that exposure to various agents generates excess Oxygen Free Radicals (OFR) in organisms. Antioxidant properties of medical plants, e.g., *Acarus*

calamus (β -asarone, major active ingredients), prevents negative effects of OFR (Manikandan and Devi, 2005) and the potential antimutagenic, antiteratogenic and antioxidant activities of these active ingredients are attributed to the presence of a relatively high percentage of phenolic compounds with high antioxidant activity (Fahim *et al.*, 1999). It can be said that the recovering effect of these plants on malformations and increasing the number of individuals may be due to the antioxidant substances that they contain.

The results indicated that selected these medical plants from Turkey may be a new medium for diet of *D. melanogaster* and source of compounds with a recovery potential against teratogenic effects. On the other hand, our results also showed that these plant extracts can be used to obtain more individuals of *D. melanogaster* for short time test methods.

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