

Investigation Healing Effects of Isgin and Dandelion against of Doxorubicin-Induced Toxicity in *D. melanogaster*

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Received: May 10, 2019

Accepted: July 15, 2019

Abstract

Chemotherapy is one of the effective methods used in the treatment of cancer. The greatest concern during the use of chemotherapeutic drugs is side effects. Increasing free radical formation in cells and tissues and thus oxidative stress is known as one of the most damaging side effects. Antioxidants used during chemotherapy may increase the efficiency of treatment by decreasing the formation of radicals due to oxidative stress and prevent healthy cells from being damaged. From this point of view, the therapeutic and protective properties of Isgin (*Rheum ribes*) and Dandelion (*Taraxacum officinale*) against toxic effects of Doxorubicin, one of the drugs used in chemotherapy, were investigated by conducting survival rate experiments on *Drosophila melanogaster*. For this purpose, in each experimental set, fly larvae (72±4 hours) were placed in the media and individuals who developed from larvae were recorded. As a result of the study, it was determined that the percentage of survival rate decreased in Doxorubicin treated group compared to the control. In addition, while the percentage of survival rates belonging to Isgin and Dandelion treated groups were higher than the control, the values in plant extracts plus Doxorubicin treated groups were close to the control. These differences in survival percentage were statistically significant ($p<0.05$). This protective effect can be explained by the inhibition of the formation of free oxygen radicals the removal of them from the biological system by the antioxidant properties of plants.

Keywords: Doxorubicin, *Drosophila melanogaster*, *Rheum ribes*, *Taraxacum officinale*, The survival rate

INTRODUCTION

Cancer is a lethal disease that occurs with an uncontrolled division of cells. According to death statistics of developed countries, cancer is the second fatal disease following cardiovascular diseases [1-2]. Chemotherapy is a standard treatment of cancer along with the surgical operation, radiotherapy, and immunotherapy [3]. Chemotherapeutic drugs used in the treatment of cancer affect tumor cells and prevent growth and proliferation of them. On the other hand, these drugs also affect normal cells in the body, leading to various side effects [4].

Doxorubicin (DOX), obtained from *Streptococcus peucetius* and also recognized as Adriamycin, is an effective and frequently used chemotherapeutic agent for various malignancies including endometrial tissue, esophagus, and liver, osteosarcomas, soft-tissue sarcomas [5- 8]. Regardless of its great antitumor efficiency, its use in chemotherapy is limited due to its varied side effects [9]. The free radical formation is mostly supposed to be responsible for DOX-induced toxicity [10]. Lipid peroxidation products such as malondialdehyde (MDA) induced by free radicals also have a contribution to toxicity [11- 12].

Natural antioxidants, which are taken up by various foods, have recently become increasingly of interest in the prevention of cancer, as well as protection from free radical damage, which is caused by toxic agents, and studies on this issue are increasing. Several natural antioxidants have been shown to alleviate the DOX-induced cell damage

without compromising its anti-tumor efficacy in the animal studies [13]. *Rheum ribes* (Isgin) belongs to Polygonaceae family [14]. It is commonly found in eastern Turkey, Lebanon, and Iran [15]. It is used for medicinal purposes and also consumed as a vegetable. *R. ribes* is a potential source of antioxidants due to including high amount of flavonoids, stilbenes and anthraquinones [16], and also considered a rich source of vitamins A, B, C, and E [17], and minerals such as aluminum, calcium, iron, potassium, magnesium, sodium, phosphorus, zinc [18]. *Taraxacum officinale* called Dandelion is widespread throughout the temperate climate regions. Traditionally, it has been used to treat inflammation and rheumatism [19]. It has also nutritive value and contains many important compounds such as terpenoid, sterol, calcium, potassium, vitamin A, nicotinic acid and vitamin C. It has been shown that leaf mixture containing *Taraxacum officinale* (Dandelion) plant increases the activity of antioxidant levels (glutathione and β -carotene) and antioxidant enzymes (superoxide dismutase, peroxidase, reductase glutathione) in rats with beneficial effects on plasma, liver, heart and kidney and lipid peroxidation [20- 21].

From this point of view, the study was carried out to investigate the therapeutic roles of the wild plants used for nutritional and therapeutic purposes against the potential toxic effects of Doxorubicin, one of the antibiotics used in chemotherapy, *Drosophila melanogaster* survival percentage experiments were carried out.

MATERIAL and METHODS

Experimental Organism

In our experiments, the larvae belonging to the Oregon line of *Drosophila melanogaster*, known as the fruit fly, were used. *Drosophila* is the ideal experimental organism [22], with short life cycles (9-10 days), large numbers of puppies, low growth rates and high human resemblance.

Survival Rate Experiments

The concentration of the chemotherapeutic agent Doxorubicin was determined to be 0.125mg/mL. Likewise, doses of *R. ribes* and *T. officinale* used as healers were determined to be 20mg/mL. Stock solutions were prepared using distilled water according to the determined concentrations. For survival rate experiments, 1-3 days virgin *Drosophila* individuals were transferred to fresh media as 5 ♀♀ X 5 ♂♂ and kept at 25±1°C and 40-60% relative humidity for 3 days. At the end of the third day, the parents were removed from the media to collect the larvae. Later, distilled water was placed in a clean petri dish and a piece was taken from the media containing the 3rd stage larvae (72±4h). For control and application groups, 100 larvae were counted. To observe the effects of Doxorubicin, *R. ribes*, and *T. officinale* separately, 2.5mL solutions of Doxorubicin, *R. ribes*, and *T. officinale* were added in the 25mL medium. In two experiment groups prepared to observe the healing effects of *R. ribes* and *T. officinale*, 2.5mL of *R. ribes* and 2.5mL of Doxorubicin were added to the first medium, 2.5mL of *T. officinale* and 2.5mL of Doxorubicin were added to the second medium. Later on, the larvae were placed in these media. The control group larvae are embedded in the Standard *Drosophila* Medium. The mouths of the bottles were covered with cotton plugs and

placed in the oven to ensure that the larvae became mature. During this process, all experimental groups were checked every day and counted for 7 days starting from the first adult flies observed in culture bottles. The data was noted twice a day by making a distinction between males and females. All experiments were repeated 3 times.

Statistical Analysis

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) 24.0 program. One way analysis of variance (ANOVA) was used to compare the survival percentages of control and experimental groups. $p < 0.05$ was taken into account in statistical evaluations. Graphs showing survival curves and individual numbers of F₁ generations were also drawn using Microsoft Windows Office-Excel program.

RESULTS and DISCUSSION

According to the results obtained from the experiment, the number of individuals matured in the control group was 74, 35 of them were male and 39 of them were female. The number of adult individuals in the experimental set applied to *R. ribes* is 80, 38 of these are male and 42 of them are female. When Table 1 is examined, the number of adult individuals in the experimental group applied *T. officinale* is 76, of which 36 are male and 40 are female. Doxorubicin treatment group had 53 adult individuals, while 25 of these individuals were male and 28 of the female. In *R. ribes* + Doxorubicin treatment group, the number of adult individuals is 65, 30 of them are male and 35 of them are female. The number of individuals emerging from *T. officinale* + Doxorubicin group is 61, 27 of these individuals are male and 34 are female.

Table 1. Effects of Isgın, Dandelion, and Doxorubicin on survival percentage of *D. melanogaster*

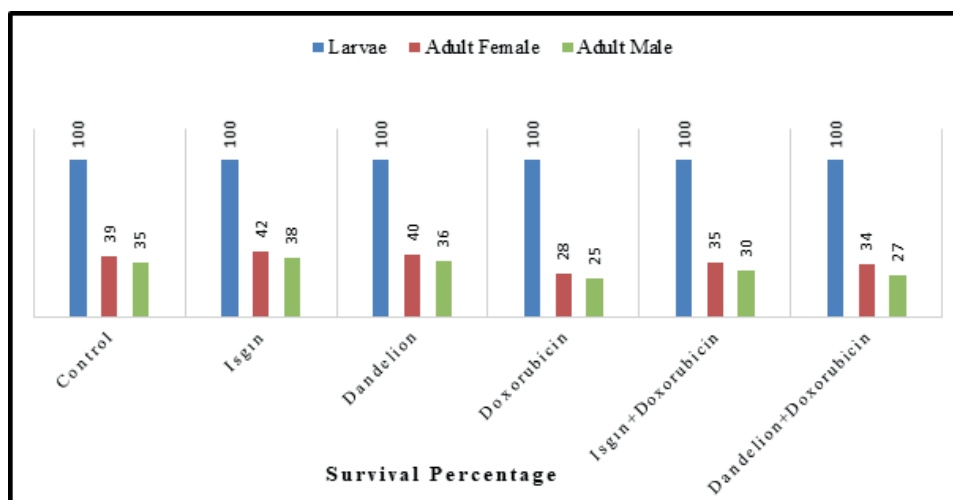
Application Groups and Concentrations	Mean Survival Rate ±Standard Error		
	♂♂	♀♀	Total
	Population	Population	Population
Control	35±0.577 ^c	39 ±0.577 ^c	74±0.577 ^d
<i>R. ribes</i> (Isgın) (20mg/mL)	38 ±0.577 ^c	42±0.577 ^c	80±0.577 ^c
<i>T. officinale</i> (Dandelion) (20mg/mL)	36±1.154 ^c	40 ±1.154 ^c	76±1.154 ^d
Doxorubicin (0.125mg/mL)	25 ±1.154 ^a	28 ±1.154 ^a	53±1.154 ^a
<i>R. ribes</i> +Doxorubicin	30±1.732 ^b	35 ±1.732 ^b	65±1.732 ^c
<i>T. officinale</i> +Doxorubicin	27±1.154 ^{ab}	34±1.154 ^b	61±1.154 ^b

a-e: Values indicated by different letters in the same column are important at $p < 0.05$.

As a result, it was determined that the percentage of survival in the experimental set exposed to Doxorubicin decreased compared to the control group. In addition, the results obtained in the experimental sets subjected to Isgın and Dandelion application were found to be more than those in the control, and in the experimental sets applied both Isgın and

Dandelion and Doxorubicin, the values obtained were close to the control (Fig 1). These differences were statistically significant ($p < 0.05$).

Fig. 1. Effects of Isgin, Dandelion, and Doxorubicin on survival percentage of *D. melanogaster*



No direct results have been found in the literature regarding the effects of Doxorubicin, *R. ribes* and *T. officinale* on *D. melanogaster*. Therefore, the results obtained from the study were evaluated in the light of related studies on different organisms in the literature. Doxorubicin is an antibiotic derivative drug used for the treatment of cancer and with side effects. Studies have shown that Doxorubicin causes significant lipid peroxidation in the heart, kidney, and liver. Lipid peroxidation is one of the most effective damages of free radicals [23]. It is accepted that Doxorubicin indirectly changes DNA strands and gene expression directly and due to free radicals, it causes changes in antioxidant enzyme activities [24].

In some studies, the effects of antioxidants, either alone or in combination with chemotherapy, have been investigated. In a study of 8500 cancer patients receiving chemotherapy, multiple antioxidant combinations have been shown to reduce side effects [25]. In a study of antioxidant vitamin supplementation in 103 children with chemotherapy, chemotherapy-induced toxicity and infection risk decreased [26]. In another study, vitamins E, A and C have been used against the oxidative stress related to chemotherapy and radiotherapy. As a result, it is seen that vitamins increased the therapeutic efficacy while normal cells have also been found to protect against apoptosis [27]. *In vitro* and animal studies have shown that the administration of free radical scavengers together with cytostatic agents such as doxorubicin and cisplatin does not reduce antitumor efficacy and that survival in antioxidant-treated animals is higher than in chemotherapy alone [28, 25].

It is seen that the results obtained from the research coincide with the literature. The toxic effect observed in the group treated with DOX is probably due to the free radicals formed. In addition, we believe that the reason that the percentages of survival in *R. ribes* + Doxorubicin and *T. officinale* + Doxorubicin treatment groups are likely to approach the control group is due to the fact that plant extracts show strong antioxidant properties.

CONCLUSION

Since the drugs used in cancer treatment, harm cancer cells as well as healthy cells, it is becoming increasingly important to search for alternative drugs are obtained from natural sources, especially plants, and have no side effects. For

this reason, alternative medicine methods based on plants have emerged [29]. In this respect, a compilation of useful information about wild plants have been tested by the public for many years is an important issue [30].

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