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Karaman İli Yöresi Bitkilerinden Arum Cinsine Ait Türlerinden *Arum dioscoridis*'in İskelet Kası Mekanik Aktivitesi Üzerindeki Yarı Maksimum Etkin Konsantrasyon (EC_{50}) Değerlerinin Belirlenmesi (Faz 0 – Preklinik Çalışma)

Determination of Half Maximal Effective Concentration (EC_{50}) Values on Skeletal Muscle Mechanic Activityof *Arum dioscoridis*, a Species Belonging to Arum Genus, One of the Plants of Karaman Province Region (Phase 0 – Preclinical Studies)

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Özet. *Arum dioscoridis* sadece Anadolu'da değil dünyanın çeşitli yerlerinde fitoterapötik olarak kullanılan bitkilerden biridir. Bu bitkinin kas mekanik aktivitesi üzerine etkileri konusunda literatürde herhangi bir çalışma bulunmamaktadır. Bu pilot çalışmada, *Arum dioscoridis*'in iskelet kasının kasılma aktivitesi üzerindeki yarı maksimum etkili konsantrasyon (EC_{50}) değerleri belirlendi. Maksimum toksik olmayan konsantrasyon (MnTK) değerine gore farklı konsantrasyonlarda hazırlanan *AD*

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ekstraktına maruz bırakılan *Wistar albino* sıçan ekstansör digitorum longus kasının mekanik tepkileri 1, 10, 20, 40, 80 ve 100 Hz frekanslarında kaydedildi. Sonuç olarak, MnTK değerinin 1/16 ve 1/32 konsantrasyonlarında kontrole gore sırasıyla 20, 40, 80 Hz ve 40, 80 Hz frekanslarında mekanik tepkide artış ve daha yüksek konsantrasyon değerlerinde bir düşüş gözlemlendi.

Anahtar Kelimeler: *Arum dioscoridis,* kas mekanik aktivitesi, $EC_{50,}$ MnTK.

Abstract. *Arum dioscoridis (AD)* is one of the plants that is used phytotherapeutically not only in Anatolia but also in various parts of the world. There is no study in the literature on the effects of this planton on muscle mechanic activity. In this pilot study, half maximal effective concentration values (EC_{50}) of *AD* on contractile activity of skeletal muscle were determined. The mechanical responses of the *Wistar albino* rat extensor digitorum longus (EDL) muscle, which were exposed to *AD* extract prepared at different concentrations according to the maximum non-toxic concentration (MnTC) value, were recorded at frequencies of 1, 10, 20, 40, 80 and 100 Hz. As a result, an increase in mechanical response was observed, at 1/16 and 1/32 concentrations of the MnTC value compared to the control in the frequencies 20, 40, 80 Hz and 40, 80 Hz, respectively, and a decrease was observed at higher concentration values.

Key words: *Arum dioscoridis*, muscle mechanical activity, EC50, MnTC.

1. Introduction

Natural plants found in nature are responsible for many known medicinal biological activities. Medicinal plants are the main source of new medicines and personal health products [28].The history of medicinal plants is probably as old as human history. Extraction and characterization of various active phytocompounds from these green factories has allowed the discovery of some high activity profile drugs [33].Increasing evidence indicates that secondary metabolites found in plants play a critical role in human health and nutrition.

Research on plants used by the public, especially in the treatment of diseases, is gaining more and more importance every day. Folk remedies used today have been tested on humans for years. In addition to those consisting of superstitions, they also have proven effects [6]. Known and accumulated knowledge of the use of herbs in healthcare systems around the world is invaluable. It must be screened, linked, explored, and strengthened [10].Therefore, the homogeneity of information on traditional medicinal uses of a medicinal plant is valuable, it needs to be evaluated using quantitative tools such as value in use and consensus partners factors[20,24].

Arum dioscoridis belongs to the Araceae family. Found in semi-arid, fallow fields and coastal areas in the Mediterranean, North Africa, and Central Asia [19]. In Turkey, it grows naturally in fields and gardens, roadsides and empty lands in the Aegean, Mediterranean, Marmara, Black Sea and Southeastern Anatolia regions [8].Archaeological evidence shows that Arum has been used by humans since ancient times [17].

These species are usually 30-50 cm high, perennial, tuberous and herbaceous plants. Its leaves are arrow-shaped, long-stalked and dark green in color. Flowers create a special inflorescence. In this case, the female flowers are at the bottom and the male flowers are at the top [7]. It is also known as "yılanpancarı", "karaabar", "kangal" and "tırşik" in the region [23].

Plants of the Arum genus have been used for centuries for nutritional and medicinal purposes, despite their toxicity. Few subspecies of this genus have been extensively explored by modern research, mainly for potential therapeutic targets and drug discovery. Other types have never been studied by available research, although some have known and well documented traditional medicinal and other uses. Many Arum subspecies have been studied so far, with the most researched *Arum dioscoridis*, *Arum maculatum*, and *Arum palaestinum* [5].

Spotted Arum is widely used in the preparation of a traditional Jordanian food item (Cha'acheel), which consists of whole wheat flour, eggs, leafy vegetables and spices cooked in boiling yogurt [4]. In the Eastern Mediterranean region, the leaves of the tırşik

plant are also eaten. Its fresh leaves and tuber contain gum, mucilage, starch, saponin and conesin. The leaves of this plant have a bitter taste, and to remove this bitter taste, the leaves are boiled, filtered and then prepared for cooking. In some regions, it is not consumed because it is poisonous [8].

The plant is also used in making a local dish called "El Gabardan Soup" in the Karaman region. It is said that those who drink the soup relax, feel better and more energetic, and those who consume a lot of it experience drowsiness.

According to the information obtained from the literature review, the plant is used against yeast, for earache [42], in the treatment of cancer and prostate diseases [1, 4, 23,27], hemorrhoids and boils [38].

The frequent use of *Arum dioscoridis* in traditional treatment methods increases the popularity of scientific studies on this plant day by day. These studies reported that the plant has antioxidant [5, 26,29, 43], anti-lipoperoxidation [25], antimicrobial [3] and enzyme inhibition [2] capacity.

The toxicity of the Arum subspecies results from several single compounds or families of compounds. Calcium oxalate is one of the main toxic compounds found in Arum plants, but it decomposes with cooking [9]. The same is true for cyano glycosides of its structure, such as triglochinin, a toxic compound found in Arum [35].

Although there is a lot of research in the literature about the plant, no study on the effect of the aqueous extract of the plant on skeletal muscles has been found in the literature. Our study on this subject is on the mechanical activity of skeletal muscles of *Arum dioscoridis* aqueous extract.

Therefore, this pilot study was conducted as a dose determination study to determine the doses at which the effects of the plant occur in an increase or a decrease direction on the mechanical activities of skeletal muscles.

2. Material and Method

2.1. Plant Examples

Species thought to be *Arum dioscoridis* belonging to the genus Arum [44], which grows between 1000 - 1600 m altitudes in the vicinity of Kalaba Village, Akcaalan, Bayir Village and plateaus located in Oyuklu Mountain, Tepeseki and Azitepe locations in Karaman province were collected in mid-spring and were identified and confirmed as *Arum dioscoridis* by the faculty members of the Department of Biology, Faculty of Science, Selcuk University.

2.2. Extraction and Isolation

Extraction and isolation procedures were carried out in Selcuk University Science Faculty Biology Department Virology Laboratory, by Selcuk University Science Faculty Biology Department faculty members. For *Arum dioscoridis* extract, the remaining parts of the dried plants were washed with deionized water and dried at room temperature. The dried plants were boiled in deionized water at a rate of 5% by weight for 15 minutes (5% by weight is generally the most acceptable ratio) [30, 32,45]. The mixture was cooled to room temperature and filtered through a Whatman filter paper (Whatman qualitative filter paper No: 1). The prepared extract was centrifuged at 2000 rpm for 5 minutes and the clear solution obtained was sealed in polypropylene tubes and cooled. Drying at room temperature was carried out in the dark [14].

2.3. Animals

Three *Wistar albino* female rats aged 3-4 months were used for the study (200-250 g). Experimental animals were obtained from Mersin University Faculty of Medicine Experimental Animals Research Unit. Pilot study permission was obtained from Mersin University Animal Experiments Local Ethics Committee for the study. (Date: 09.03.2020, Protocol Number: 52602694-05.01.04 1332825)

2.4. Application Doses *Arum dioscoridis*

The doses to be applied to the tissue samples obtained from the experimental animals were calculated according to the maximum non-toxic concentration (MnTC) value obtained in a previous our study [13]. As a result of the cytotoxicity test performed with the XTT method, the maximum non-toxic concentration (MNTC) value of the *Arum dioscoridis* was 1561 µg/ml. This value was also used in now study.

In this study, groups were divided into 6 groups (A1, A2, A3, A4, A5 and A6). According to this, the groups: Control (Only Kreps solution applied; (in mM): NaCl 118, KCl 4.8, CaCl2 2.5, MgSO4 1.2, NaHCO3 24, KH2PO4 1.2, glucose 11 and at pH 7.40); Dose-1 (applied by dissolving the extract in Krebs solution with MnTC value-A6), Dose-2 (applied by dissolving the extract in solution with MnTC/2 value-A5), Dose 3 (applied by dissolving the extract in solution with MnTC/4 value-A4), Dose 4 (applied by dissolving the extract in solution with MnTC/8 value-A3), Dose 5 (applied by dissolving the extract in solution with MnTC/16 value-A2), Dose 6 (applied by dissolving the extract in solution with of MnTC/32 value-A1).

2.5. Recording Technique of EDL Muscle Mechanical Responses

Isolated organ bath (Isolated Organ Bath Stand Set-IOB S99) and isometric force transducer (FDT 05 Force Displacement Transducer) were used to record EDL muscle mechanical responses. The isometric force transducer output was connected to the amplifier module (MAY-GTA-200) in the analysis and recording system (BIOPAC MP160 Systems Inc., USA). Organ bath filled with Krebs solution. The EDL muscle was placed between two platinum wire electrodes so that the electrodes were in contact with the tissue. The bath solution was kept at 37 °C with a temperature controlled heating circulator (Heating Circulator/Model MAY WBC 3044-PR) and was also continuously gassed with a mixture of 95% O_2 and 5% CO_2. The Krebs Solution in which the EDL muscle was placed was changed every 15 minutes and the muscle was incubated in this medium for 30 minutes to equilibrate it by adapting to the bath environment [22, 37, 41]. After the equilibration period in Krebs Solution, the preload value that brought the EDL muscle to optimal length (L0) was measured. In addition, before each stimulation protocol

was applied, appropriate preload values were adjusted with a micrometer attached to one end of the muscle, which brought the muscle preparation to the optimal length. The maximum stimulation output of the stimulator in the analysis and recording system was ± 5 V. It was found that this value is not an appropriate range for the supramaximal stimulus voltage required to induce the muscles. Therefore, the stimulator (STM 100-EXT STIM) on the analysis and recording system was connected to a stimulus isolator (MAY-ISO150-A Serial No: 200.001–1 Stimulus Isolated Power Supply) adjustable up to 150V output voltages. Preliminary trials were then performed. Twitch pulse (Tw-1 Hz) was adjusted to have duration of 0.5 ms and amplitude of supramaximal intensity. After preliminary tests were completed, it was detected that the mechanical activity of EDL muscle reached the supramaximal amplitude with approximately between 60–70V stimulation intensity.

For control and all dose groups, stimulations were given at different frequencies (Tw, 10, 20, 40, 80 and 100 Hz) with 5 minutes intervals for the EDL muscles brought to the optimal length and the mechanical responses of the muscle (For dose groups solution with extracts were replaced after every records). The mechanical responses of the EDL muscle were recorded in the data recording and analysis system, which transferred the responses transmitted through the isometric force transducer to the computer via the difference amplifier.

2.6. EC_{50} Value Determination

Half-maximum effective concentration (EC_{50}) refers to the concentration of a drug, antibody, or toxic substance that causes a response halfway between the minimum and maximum values obtained after a certain exposure time. More simply, EC_{50} can be defined as the concentration required to achieve %50 effect [34]. To calculate the EC_{50} value of rat isolated skeletal muscle contraction force exposed to *Arum dioscoridis* aqueous extract, contraction forces were calculated as % contraction for each applied frequency stimulus (The maximum contraction force was evaluated as 100% and other contraction forces were determined as % contraction by proportioning to the maximum contraction). The % contraction-concentration graphs was drawn using the OriginPro8

program for each applied frequency stimulus (Tw, 10, 20, 40, 80 and 100 Hz). Concentration values corresponding to 50% contraction were read on the graphs and frequency dependent EC_{50} values were determined.

3. Results

3.1. EDL Muscle Mechanic Activity Findings

3.1.1. Preload Values That Bring EDL Muscle to Its Optimum Length (L0)

Table 1 shows the preload values applied to the muscle strip while determining the optimum length at which the EDL muscle will generate maximum force, and the sample data series for the force responses of the muscle at these preload values. A square pulse of 60 V amplitude and 0.5 ms duration was applied each time to generate the force response in the muscles brought to their optimum length.

As seen in the table 1, until the maximum force is created in the muscle, the amount of preload applied is increased, and the data before the value where the force response starts to decrease in return for the increase in the preload is used as the appropriate preload value for that muscle strip. The preload value, which brings the EDL muscle to the optimum length to create the maximum contraction force, was determined as 2.6 g.

Table 1.

Sample data for preload (g) values and force responses applied to a muscle strip through a micrometer, which will bring the muscle to its optimum length at which it will generate maximum force.

Applied preload (g)	**The resulting force response (g) for 60V (maximal)**	**The resulting force response (g) for 70V (supramaximal)**
2,4	10	10
2,6	11	11
2,7	9	9
2,8	9	9

3.1.2. The force–frequency relationship

The force responses to the stimulus obtained by applying stimulus at different frequencies (Tw, 10, 20, 40, 80 and 100 Hz) at 5-minute intervals to the EDL muscle, which has been brought to the optimum length, are given in Table 2.

At frequencies of Tw, 10 and 100 Hz, a decrease in the contraction forces recorded at all concentrations was observed compared to the control.

The maximum contraction (g) – Stimulus frequencies (Hz) graphs obtained using the data in Table 2 are given in Figure 1 and Figure 2.

Table 2.

For control and all dose groups values of concussion strengths obtained for single stimulus (Tw) and stimuli at different frequencies in muscle mechanical activity

	Control(g)	**A1(g)**	**A2(g)**	**A3(g)**	**A4(g)**	**A5(g)**	**A6(g)**
T_w	12,83	11,05	11,74	9,75	7,41	6,87	2.52
10 Hz	21,74	13,69	14,77	11,78	8,89	8,03	2,00
20 Hz	51,85	51,04	51,93	45,37	32,82	26,60	2,30
40 Hz	63,83	66,56	67,85	57,89	48,37	32,31	2,25
80 Hz	83,47	84,00	85,13	60,52	63,80	34,73	2,26
100 Hz	84,76	84,54	82,63	64,32	49,65	30,95	1,87

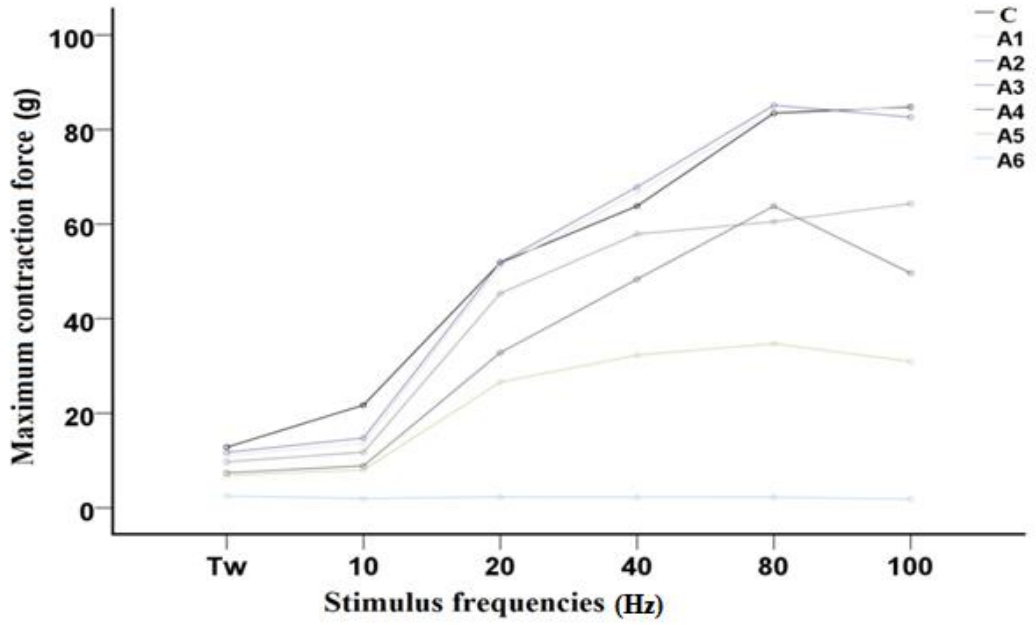

Figure 1. For control and all dose groups, recordings of maximum contraction force curves of the EDL muscle at different stimulation frequencies for mechanical activity in line chart.

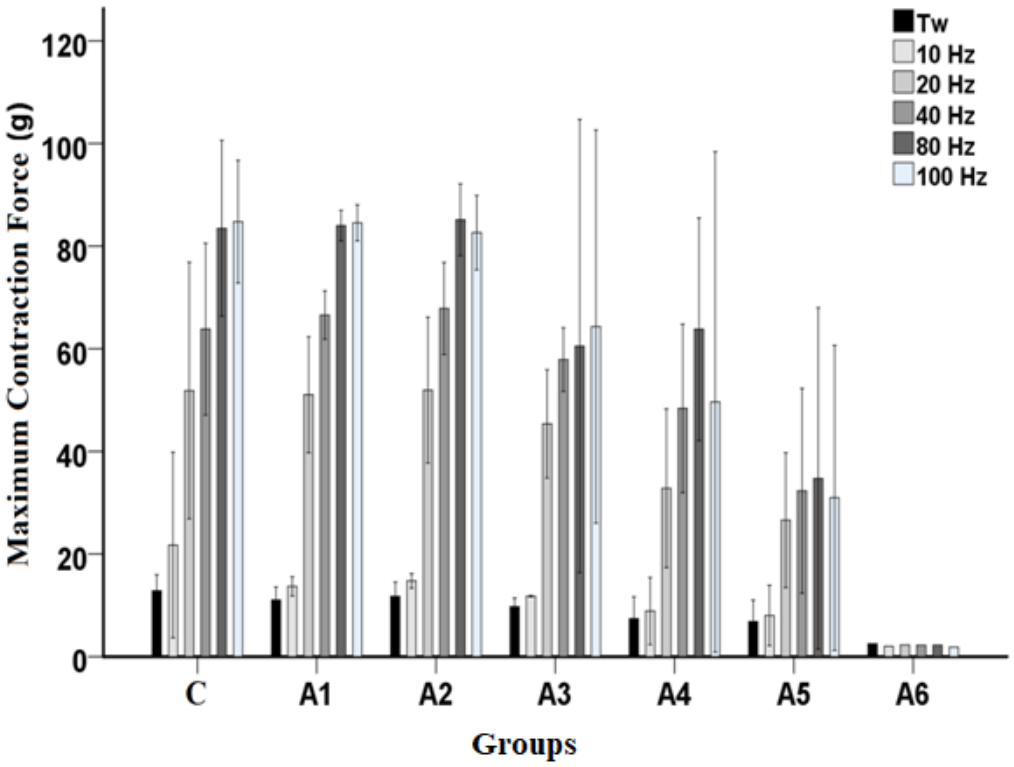

Figure 2. For control and all dose groups, recordings of maximum contraction force curves of the EDL muscle at different stimulation frequencies for mechanical activity in column chart.

The mechanical contraction responses of the EDL muscle to stimuli at different frequencies (respectively at TW, 10, 20, 40, 80 and 100 Hz) are given in the Figure 3, Figure 4, Figure 5, Figure 6, Figure 7 and Figure 8.

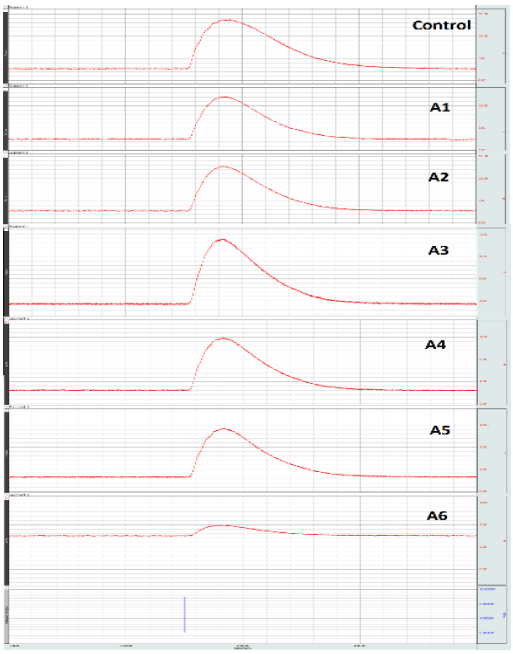

Figure 3. For all groups, recordings of muscle mechanical activity (TW)

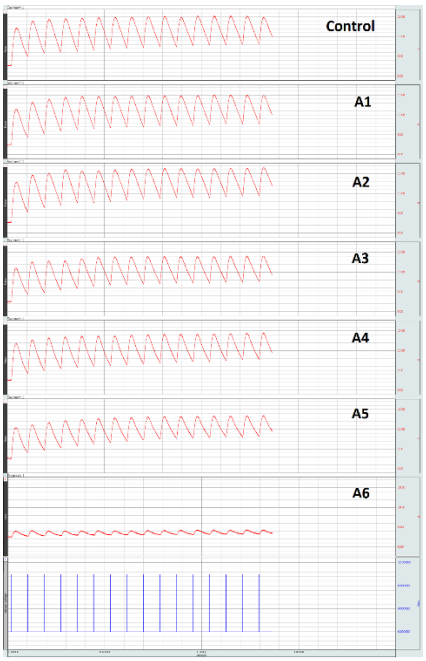

Figure 4. For all groups, recordings of muscle mechanical activity (10 Hz)

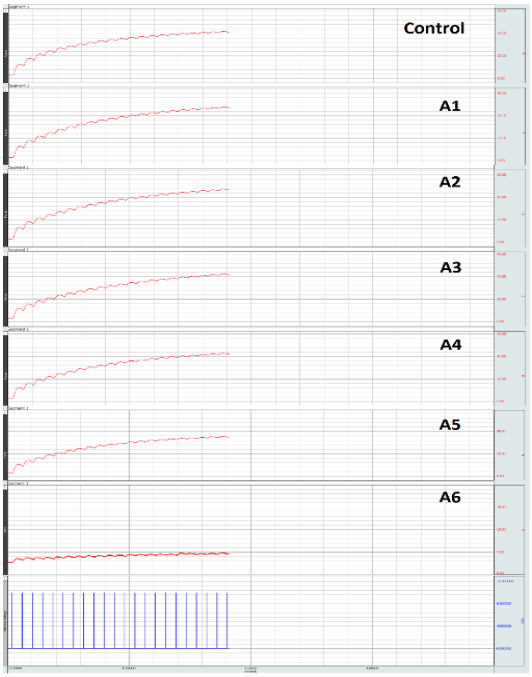

Figure 5. For all groups, recordings of muscle mechanical activity (20 Hz)

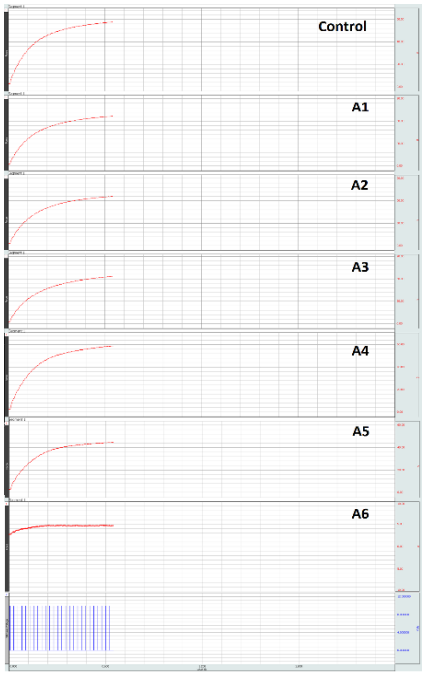

Figure 6. For all groups, recordings of muscle mechanical activity (40 Hz)

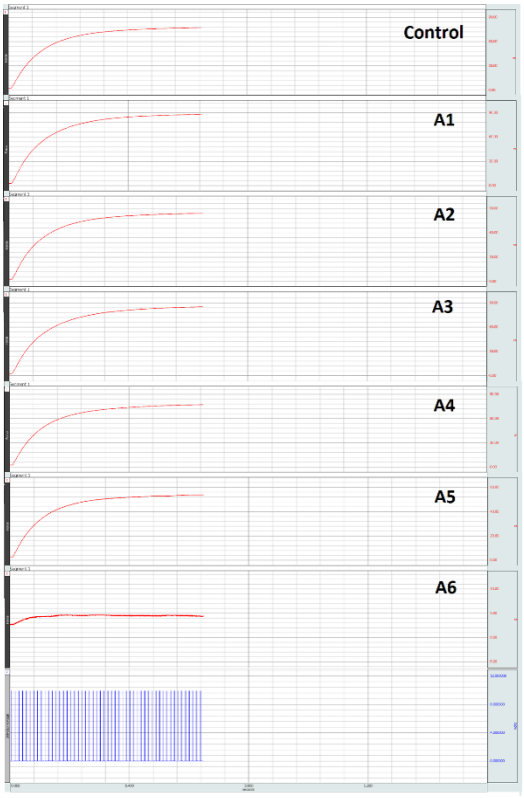

Figure 7. For all groups, recordings of muscle mechanical activity (80 Hz)

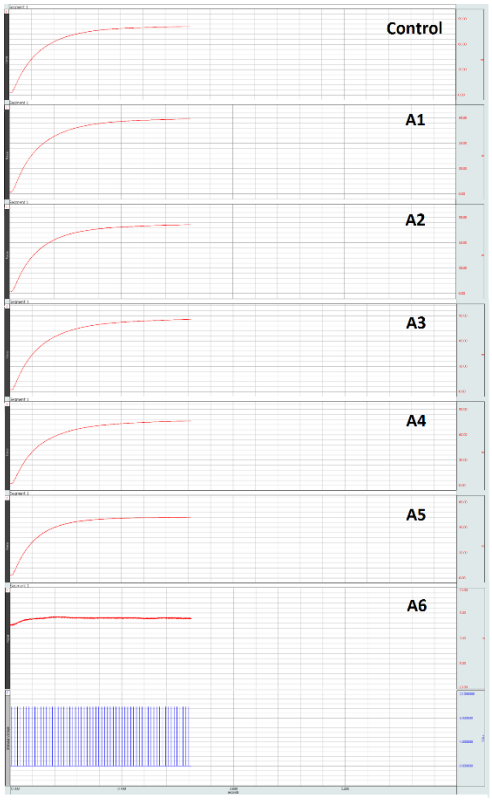

Figure 8. For all groups, recordings of muscle mechanical activity (100 Hz)

3.1.3. The Contraction Force-Concentration Relationship

Contraction force (g) – concentration (µg/ml) graph (Figure 9) was drawn to determine the effects of *A. dioscoridis* on skeletal muscle contraction force (for Tw, 10, 20, 40, 80 and 100Hz).

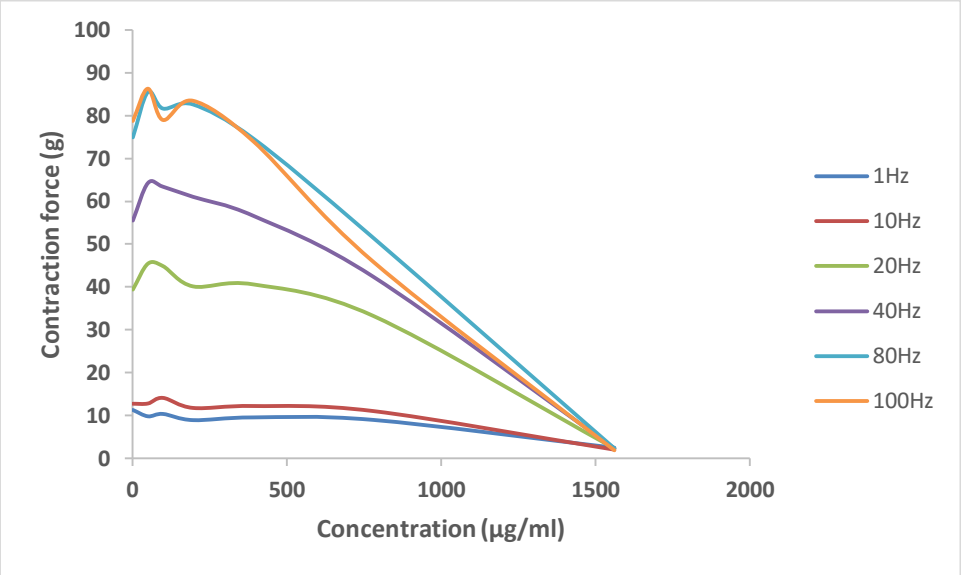

Figure 9. Effects of *A. dioscoridis* on muscle contraction force. Graphs showing contraction forces-concentration curves for all frequencies (for Tw, 10, 20, 40, 80 and 100Hz)

3.2. EC_{50} Values

EC_{50} values were recorded from the frequency dependent % contraction force - concentration graphs drawn with OriginPro8 and results were given in Table 3. For all stimulus frequencies (Tw, 10, 20, 40, 80 and 100Hz), EC_{50} values are shown in contraction (% g) –concentration (µg/ml) graphs in Figure 10, Figure 11, Figure 12, Figure 13, Figure 14 and Figure 15 (respectively for Tw, 10, 20, 40, 80 and 100Hz).

Table 3.

EC_{50} values by stimulus frequencies

	Tw (Hz)	**10 (Hz)**	**20 (Hz)**	**40 (Hz)**	**80 (Hz)**	**100 (Hz)**
EC_{50} (µg/ml)	1197,06	1130,35	1062,77	999,96	929,34	827,11

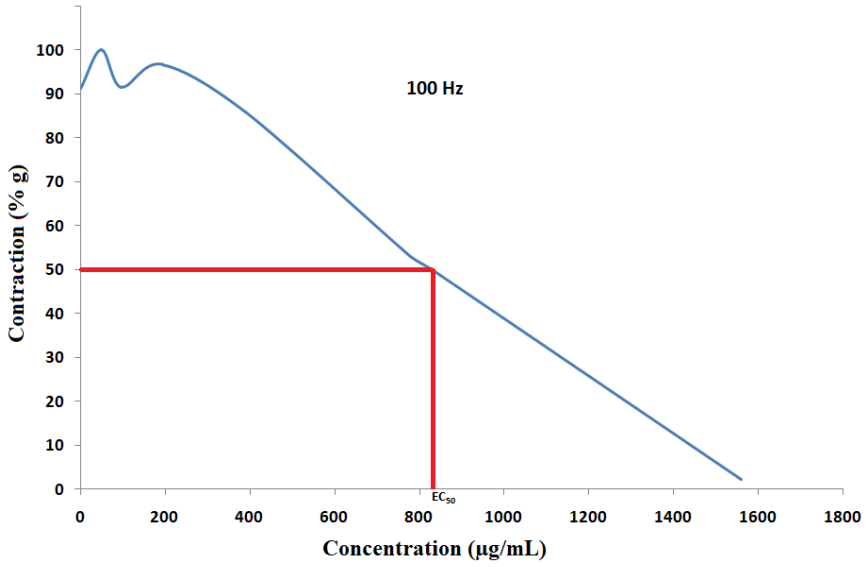

Figure 10. EC_{50} value on the % contraction-concentration graph for all groups (For 100 Hz)

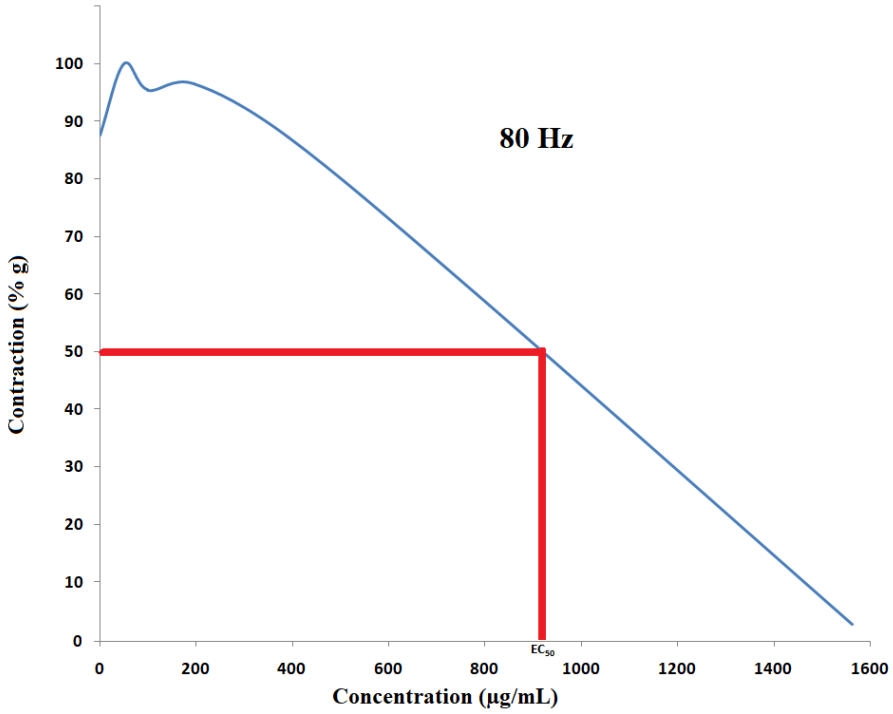

Figure 11. EC_{50} value on the % contraction-concentration graph for all groups (For 80 Hz)

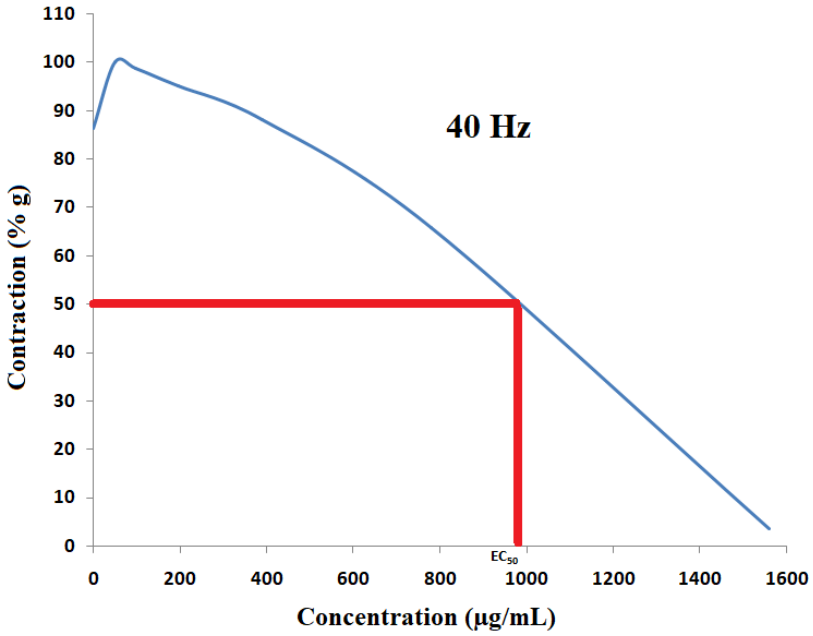

Figure 12. EC_{50} value on the % contraction-concentration graph for all groups (For 40 Hz)

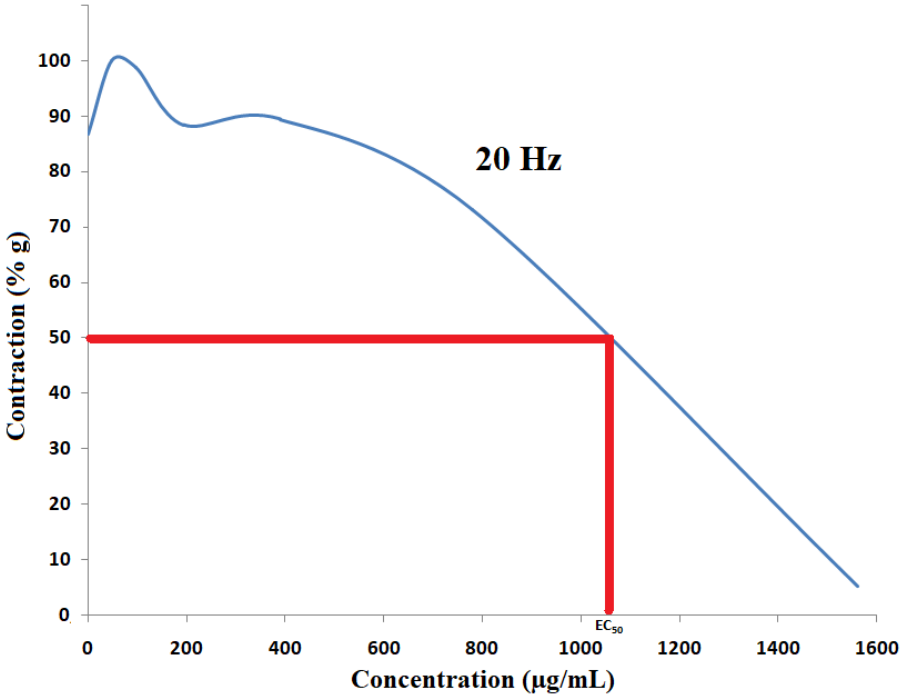

Figure 13. EC_{50} value on the % contraction-concentration graph for all groups (For 20 Hz)

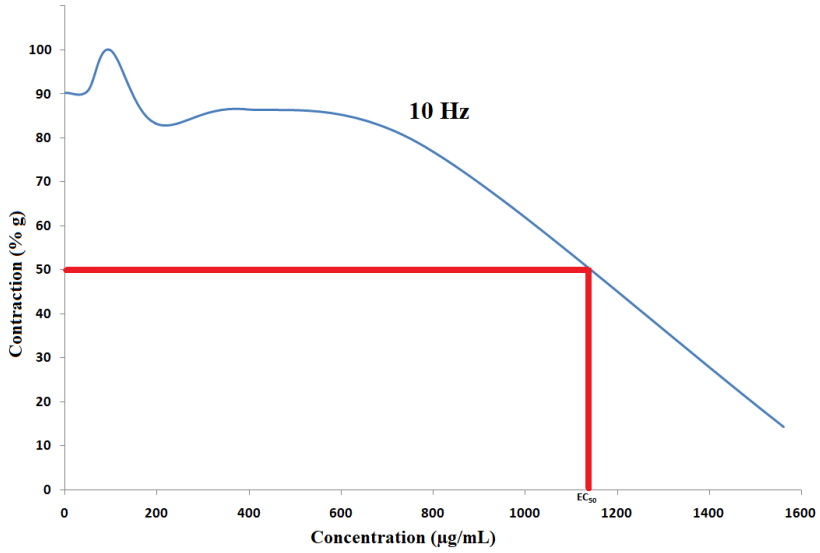

Figure 14. EC_{50} value on the % contraction-concentration graph for all groups (For 10 Hz)

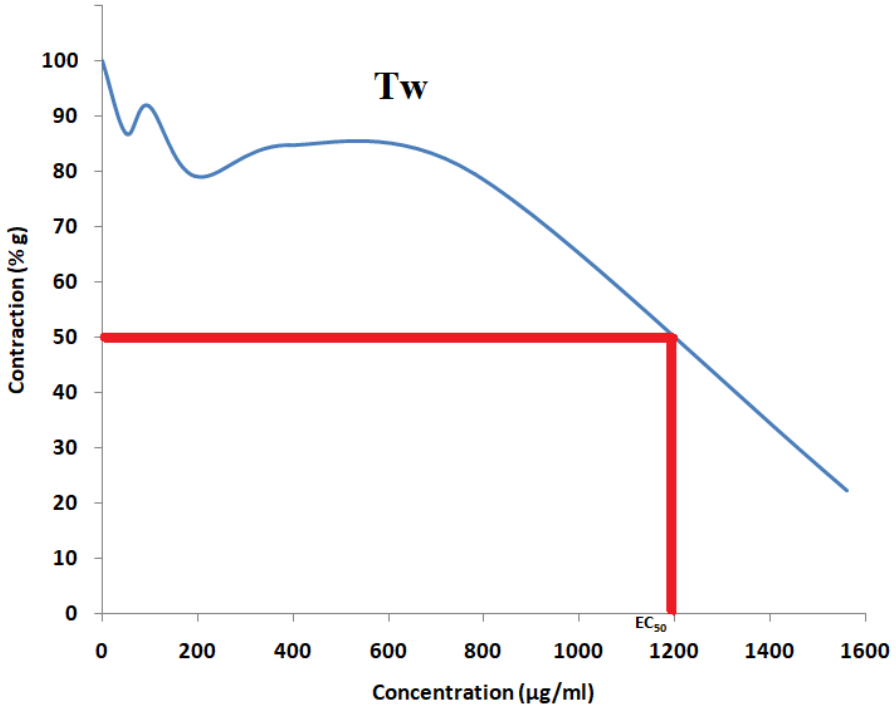

Figure 15. EC_{50} value on the % contraction-concentration graph for all groups (For Tw)

4. Discussion and Conclusion

Since plants are living organisms, all of the chemical reactions that provide the matter and energy necessary for this organism to live constitute metabolism. Plants synthesize a wide range of organic compounds, generally classified as primary and secondary metabolites. Primary metabolites are compounds that have essential roles in photosynthesis, respiration, growth and development. Examples of these compounds are phytosterols, acyl lipids, nucleotides, amino acids, carbohydrates and proteins. Other phytochemicals that can accumulate in surprisingly high concentrations in some plants are secondary metabolites. Secondary metabolites, which show many structural differences, are distributed in the plant world in a way that can be diagnostic in chemotaxonomic studies [11,12]. Secondary metabolites, considering their biosynthetic origins, are divided into four main groups as phenolic compounds, terpenoids, nitrogen-containing alkaloids and sulfur-containing compounds.

Ethnopharmacology and drug discovery from natural products remain important topics for the current target. According to the World Health Organization, a great majority of the world's population rely on traditional treatments (mostly herbs) for their health [46]. In fact, herbs/plants are humanity's oldest friends. They are not only provided food and shelter, but also served humanity as a cure for different diseases [31]. Medicinal plants have provided modern medicine with a multitude of herbal medicinal therapeutics [18]. These drugs are either all-natural extracts or semi-synthetic substances derived from natural precursors or model-developed substances (prototype). Aspirin, atropine, artimenin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine and vinblastine are some of the products that medicinal plants have given us. Many of these plant-derived medicines were first discovered by studying traditional treatments and folklore of the indigenous people. Despite the great progress in synthetic chemistry, some of these have not been substituted. Few drugs derived from plants have had any significance in traditional medical practice over the past few decades, despite growing interest in phytomedicine. Basically, ethnopharmacology has already played an important role in the development of traditional medicine and is likely to play a more important role in the years to come [21].

Arum dioscoridis is a plant that has been used extensively in traditional treatment methods in many parts of the world from past to present. The fact that the plant is so popular results in an increase in the number of studies on it. Antilipoperoxidation, antiviral, antibacterial, antifungal, anticancer effects have been shown in scientific studies based on the use of the plant in traditional treatment methods, and it has been reported that its antioxidant and enzyme inhibitory capacity is high [2, 3, 5, 25, 26, 29, 36, 43]. In addition, studies have shown that its content is quite rich in minerals, flavonoids, alkaloids, phenolics and calcium oxalate [2, 9, 15, 16, 35, 39, 40, 43, 47].

In this study, the effective dose range of the aqueous extract of the *Arum dioscoridis* plant, which is used both as a nutrient and phytotherapeutic in traditional folk medicine in our country and in many parts of the world, on skeletal muscles was investigated. Although there are many studies on the interaction of *Arum dioscoridis* with biological tissues in the literature search, no study on striated muscles has been found. Isometric contraction parameters were used to determine the effects of *Arum dioscoridis* aqueous extract on EDL muscle biomechanics at the intensity, frequency and exposure time we used. For this purpose, the isometric contraction curves of the muscle responses were analyzed by stimulating the EDL muscle with maximal electrical pulses and the mechanical properties of the muscle were investigated.

As a result of the mechanical activity recordings, an increase was observed in the mechanical activities of the EDL muscles exposed to solutions of 1/32 and 1/16 concentrations of the MnTC value, at 20, 40 and 80 Hz frequency stimuli. A decrease was observed at other frequencies and concentrations. In the solution with MnTC value, the mechanical activity dramatically decreased. In addition, EC_{50} values were calculated for all excitation frequencies. The values found are between 50% and 65% of the MnTC value. So, MnTC value of the plant extract has an inhibitory effect on skeletal muscle at concentrations of 50% and above.

These results show that while the aqueous extract of *Arum dioscoridis* contributes to the muscle mechanical activity at certain concentrations, it suppresses the mechanical activity at certain concentrations.

EDL muscle dissected from adult female *Wistar albino* rats was exposed to different concentrations of *Arum dioscoridis* aqueous extract adjusted according to MnTC value. The effective dose values on the skeletal muscles of the plant used as food and in traditional treatment methods in certain regions were investigated. In the literature review, no study examining the effects of this plant on skeletal muscles was found. For this reason, this pilot study can form a basis for studies that will cover the effects of *Arum dioscoridis* on skeletal muscles. We hope that the results of our pilot study can guide studies on the functional and morphological properties of muscles.

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