INVESTIGATION OF THE TOXIC EFFECT OF *MANDRAGORA AUTUMNALIS* (MANDRAKE) EXTRACTS ON *DAPHNIA MAGNA*

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Abstract. Fish farming is a very important commercial sector in Türkiye. The fish produced are exported to abroad rather than the domestic market. The presence of toxic residues in these fish produced for export reduces their commercial value. The use of synthetic anesthetics causes residues in fish to lose their commercial value. The aim of this study is to solve a commercial problem if the mandrake plant is used as an anesthetic in fish instead of synthetic anesthetics that create toxic residues as anesthetic. Since the toxic and lethal doses of mandrake to be used have not been studied before, a new field will be opened with this study. As a result, it was concluded that mandrake plant can be used as an anesthetic. As a result of the test, it was observed that the death rate of *Daphnia magna* using the extracts prepared by the maceration method was higher than that by the other methods. The highest LC50 value was obtained in the maceration method with 55% concentration and the lowest LC50 value was obtained in the microwave method with 100% concentration. Since the organic matter obtained by the maceration method is at a higher level than that by the other methods, the mortality rate was also higher. As a result, it was concluded that mandrake plant can be used as an anesthetic.
Keywords: *Mandragora autumnalis, adamotu, Daphnia magna, fish, anesthetic agent*

Introduction

In aquaculture, especially in some vaccination applications made in different periods of farmed fish, handling of fish during spawning induction and grading processes of the brood fish causes stress. Since fish live in a dynamic environment, they have the ability to adapt quickly and effectively to the stress caused by sudden environmental changes. However, there is an energy cost in this adaptation process, defined as a loss in growth. Adaptation is effective in the short term and increases the chances of survival of the fish. If the stress continues in a chronic state, after a while the physiological adaptation will be impaired and the fish will go into a state of tolerance and become completely vulnerable to disease and other shocks. For this reason, anesthetic agents are used to prevent stress. Anesthetics or sedatives are used for many purposes such as calming and immobilizing fish, handling, examining, catching, transporting, spawning, measuring and vaccinating fish (Yanar and Genç, 2004; Çetinkaya and Şahin, 2005; Serezli et al., 2005; Hajek et al., 2006). The most commonly used synthetic chemicals for anesthetic purposes in our country generally cause residues in fish products, thus have negative effects on human health (Yıldırım et al., 2009). Therefore, there is a need today for natural products that are relatively safe for humans and animals. Due to the importance of natural herbal applications instead of synthetic chemical anesthetic drugs, the discovery and use of herbal-containing anesthetics in fish has become widespread with the research studies in recent years carried out in recent years. Preclinical studies are carried out for the detection of herbal medicines (Öğüt, 2005).

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Mandragora genus is represented by two species in Turkey as M. autumnalis and Mandragora officinarum (Güner et al., 2012). Mandragora autumnalis is known by different local names such as ground loquat around Silifke, Mersin, dog apple around Kalkan, Kınık, Antalya, human grass, blood-drying (Baytop, 2007). Mandragora autumnalis BERTOL (Solanaceae family) is synonymous with M officinalis MILL (Arbia et al., 2019). The existence of mandrake dates back thousands of years and has been used for surgical anesthesia throughout the 15th century (Ramoutsaki et al., 2002; Chidiac et al., 2012). Different parts (root, fruit, and leaves) of Mandragora species have been used therapeutically for many pains (including pain, insomnia, eye diseases, inflammation, and ulcers) in ancient times (Zohary, 1982). Various studies have demonstrated the phytochemical profile (alkaloids, essential oils, etc.) of all Mandragora species (Uysal et al., 2016) including M. autumnalis (Bekkouche et al., 1994; Nutton, 1996; Von Hintzenstern, 1989; Al-Khalil and Alkofahi, 1996).

Daphnia spp. is an indispensable food source for freshwater fish in nature. These species are also used as live bait by fish breeders (Steffens, 1981; Vijerberg, 1989). Daphnia spp. are freshwater crustaceans containing high protein and essential fatty acids. Although its nutritional value varies according to age and species, approximately 50% of its dry weight consists of protein. Fatty acids in adults are higher than in young individuals. It also has a distinguished concentration of vitamins A and B. Therefore, they form a quality and nutritious feed for fish (Akyıldız, 1992; Cirik and Gökpınar, 2006). It was stated that Daphnia species have an important place in determining the water quality, and generally good results are obtained in water taken from natural environments (Şanal and Köksal, 2005). Excess inorganic nutrient content can be toxic to zooplankton (Vijverberg, 1989).

Various plants are used as natural and organic anesthetics in fish. Although reports of the use of clove oil as an anesthetic in fish date back to many years (Endo et al., 1972), its use as a potential fish anesthetic has increased in recent years (Soto and Burhanuddin, 1995; Keene et al., 1998; Cho and Heath, 2000; Wagner et al., 2003; Kanyılmaz et al., 2007; Gullian and Villanueva, 2009; Sudagara et al., 2009; Zahl et al., 2009; Imanpoor et al., 2010; Akbulut et al., 2011a, b; Dolezelová et al., 2011; Akbulut et al., 2012; Yıldız et al., 2013). Clove oil has become an anesthetic for fish because it is well tolerated by fish, has a short cleaning time, is relatively safe, and is cheap (Kanyılmaz et al., 2007). In addition to clove oil, peppermint oil, methyl salicylate oil, rosemary oil (Ghazilou and Chenary, 2011; Roohi and Imanpoor, 2015), Zataria multiflora (Sharif Rohani et al., 2008), Lippia alba, Ocimum gratissimum, Aloysia triphylla and Hesperozygis ringens (Cunha et al., 2010; Silva et al., 2012, 2013; Parodi et al., 2014; Gressler et al., 2014) essential oils were found and studies were conducted on their anesthetic effects. In the experiment on goldfish, it was determined that peppermint oil and lavender oil could be used safely as anesthetics (Küçükosman, 2019).

In this study, the essential oil and other extracts of *M. autumnalis* plant grown in Turkey were analyzed with GC/MS (Gas Chromatography-Mass Spectrometer) device.

In order to determine the toxicological effect of extracts of different concentrations, an acute toxicological test was performed on Daphnia (daphnia) grown in the aquarium and the lethal concentration (LC 50) was determined as a result of the test.

The aim of this study is to solve a commercial problem by using mandrake plant as a fish anesthetic by preventing the formation of this toxic residue. Since the toxic and lethal doses of mandrake to be used have not been studied before, a new field will be opened with this study.

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Materials and methods

In the study, *M. autumnalis* plant collected from Silifke and Mersin was used. The plants obtained from the people who collect mandrake from nature were identified in the plant identification laboratory of Harran University and archived with the number 6376 (*Fig. 1*). Extractions from plants were carried out in the Harran University Central Research Laboratory.

Figure 1. *M. autumnalis (Mandrake) plant*

Daphnia magna culture and nutritional conditions

Fresh water with known physicochemical parameters was used for *D. manga* culture (*Table 1*). The culture was established in 35 × 15 cm glass aquariums with a water level of 40-50 cm. A 16-h light and 8-h dark photoperiod was applied to Daphnia culture. White fluorescent light was used for illumination. The culture was constantly aerated with an aeration engine. The pH and dissolved oxygen of the culture were monitored once a week, and the water temperature was monitored daily.

The population density was adjusted to 1 individual in 2 ml of water. When this density was exceeded, some of the adults were transferred into another aquarium.

Extraction of the plant samples

In this study, the lethal concentration value (LC50) of solvents prepared in 3 different polarities (N-Hexane, Water 50% + ethanol 50% and water solvents) to be used in extraction on Daphnias was investigated. Since hexane and ethanol components cause sudden death of Daphnia, it is not preferred to be used in extraction. Extracts obtained with water were used to determine the lethal dose.

Aqueous extraction method

Three different methods were used for the preparation of the *M. autumnalis* extracts for aqueous extraction: maceration, conventional and microwave extraction methods. In each method, 16 g of product and 100 ml of distilled water were used.

In the extraction process by maceration, 16 g of the sample was weighed. 100 ml of distilled water was added to the sample and it was left at room temperature, in the dark, for 24 h with the lid closed. The purpose of this extraction is to extract the secondary

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metabolites of mandrake, which is a natural product, without being affected by heat and light environment and without deterioration.

In the traditional extraction process, 16 g of the sample was weighed and 100 ml of distilled water was added to it. Then, the extracts were extracted by boiling for 1 h in the extraction device.

Table 1. *Physico-chemical properties of the dilution water used in the experiment*

Ph	7.9
Conductivity (µs/cm at 20°C)	72
Fe (mg/L)	0.02
Mn (µg/L)	1
Cl (mg/L)	0.5
Al (µg/L)	2.39
Na (mg/L)	1.74
K (mg/L)	1.1

In the microwave assisted extraction process, 16 g of the product was weighed and 100 ml of distilled water was added. It was extracted at 250-W microwave power for 1 h. The main purpose of the microwave extraction process is to extract the secondary metabolites, which are antioxidative and antiseptic, in the sample without deterioration.

As a result of the processes, the product obtained was filtered with Wattman filter paper and the obtained extracts became ready for use. The used methods are shown in *Table 2*.

Table 2. *Used extraction methods*

	Maceration	**Traditional**	**Microwave**
Duration	24 h	1 h	1 h
Temperature	Room temperature		250 W

Experiment implementation

In the study, 5 ml samples were prepared at different rates for all three extractions. Experiments were carried out in four repetitions with 5 test and 1 control groups for each station and 10 Daphnia in each group (10 individuals/5 mL). Samples were prepared at 10%, 30%, 50%, 70%, 90% and 100% concentrations.

Proportions of used extracts

For 10%: 4.5 ml of aquarium water of Daphnias was added to 0.5 ml of extract prepared in advance, and it was completed to 5 ml.

For 30%: 3.5 ml of aquarium water of Daphnias was added to 1.5 ml of extract prepared in advance, and it was completed to 5 ml.

For 50%: 2.5 ml of aquarium water of Daphnias was added to the previously prepared 2.5 ml of extract and it was completed to 5 ml.

For 70%: 1.5 ml of aquarium water of Daphnias was added to the previously prepared 3.5 ml of extract and it was completed to 5 ml.

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For 90%: 0.5 ml of aquarium water of Daphnias was added to 4.5 ml of extract prepared in advance, and it was completed to 5 ml.

For 100%: 5 ml of the prepared extract was used.

Toxicity tests

Before the acute toxicity tests, range finding studies were carried out to determine the effective concentration range for each station. The range of the lowest concentration with 100% mortality and the highest concentration with 0% death (no death) was determined.

Acute toxicity tests were performed on a static system basis in accordance with the "Daphnia sp. Acute Immobility Test" standard protocol (OECD, 2004; Test No: 202). During the experiment, the test solutions were not changed, the test vessels were not aerated, and the organisms were not fed.

Experiments were performed in four repetitions, with five test and one control group for each station, with 10 Daphnia in each group (10 individuals/5 ml). A control group was used, which was run in conjunction with the conditions under which the experiment was conducted. No herbal extracts were added to the control group.

The experiment was set up by providing optimum living conditions of the organisms in the laboratory. The experiments were carried out in a photoperiod of 16 h light / 8 h dark and at a water temperature of 20 ± 1°C. Adequate *D. magna* was provided by ensuring their reproduction and proliferation in this environment. Falcon tubes were used in the study. Daphnias were added to the prepared medium by diluting the prepared mandrake extract in the tube at the desired rate. By observing the movements of Daphnia in the tubes at equal times, the lethal concentration ratio was tried to be determined. The number of Daphnia that died in the tubes and the time of death were noted in detail.

Gas chromatography-mass spectrometer (GC/MS)

The essential oil component analysis of the samples was carried out by Mass Detector Gas chromatography (GC-MS) Shimadzu brand QP2020 model device using a capillary column (RXI-5MS; 30.0 m × 0.25 mm × 0.25 μm). In the analysis, helium was used as the carrier gas at a flow rate of 3 ml/min. Samples were injected into the device as 1 μl with a split ratio of 10:1. The injector temperature was kept at 250°C. The column temperature program was set to be 40°C (2 min), 40°C to 240°C, 3°C/min and 240°C (2 min). In line with this temperature program, the total analysis time was 70 min. Scanning range (m/z) of 35- 600 atomic mass units and electron bombardment ionization of 70 eV were used for the mass detector. WILEY NIST W9N11 library data is based on the identification of essential oil components.

Results

According to the results, it was observed that the death rate of Daphnia in the extraction prepared by the maceration method was higher than the traditional and microwave method. This was also evident in the LC50 values. The highest LC50 value was obtained in the maceration method with 55% concentration and the lowest LC50 value was obtained in the microwave method with 100% concentration. It has been determined that the extract of mandrake obtained by all methods has a lethal effect on D. magna in a short time (in 30-60 min) and even at very low concentrations.

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In the first results, it was observed that the death rate of Daphnia with the extraction prepared by the maceration method was higher than the traditional and microwave method.

It was determined that Oct-7-enol < 3,7-dimethyl- > component, which is one of the volatile components detected in the analysis of Mandrake by Gas Chromatography-Mass Spectrometer (GC/MS - Shimadzu), has allergic effects and is harmful to the aquatic environment. Although there is no literature on the effects of Dodecane and Butyraldehyde < 2-methyl- > components in the aquatic environment, it has been found to have a toxic effect on laboratory animals. It is considered that the lethal effect on *D. manga* is due to Oct-7-enol < 3,7-dimethyl- > and Dodecane and Butyraldehyde < 2-methyl- > components, and a larger study is needed in this regard (*Table 3*).

Table 3. *Major volatile component composition of Mandragora autumnalis*

Peak	R. Time	Area	%	Name
1	57	1490262	19.15	Citronellyl propionate
2	57	1393143	17.91	Phytol
3	57	1326835	17.05	Farnesol < cis,cis- >
4	41	645524	8.3	Docosane
5	31	490620	6.31	Heptadecane
6	44	412625	5.305	Pentacosane
7	43	309466	3.98	Octadecane
8	20	262244	3.37	Pelargonaldehyde
9	56	230594	2.96	Geranyl propanoate
10	20	197374	2.54	Dodecane
11	56	189850	2.44	Oct-7-enol < 3,7-dimethyl- >
12	56	170894	2.2	Nerylisovalerate
13	35	155349	2	Hexadecane
14	9	93089	1.2	Butyraldehyde < 2-methyl- >

Discussion

Al-Maharik and others (2022) in their research documented for the first time the chemical components of the ripe fruit flavonoid fraction (FFM) of M. autumnalis and evaluated its antidiabetic, antiobesity and antimicrobial effects. The results show that FFM significantly increases the amount of GLUT4 in PM both in the presence and absence of insulin. These findings suggest that FFM has anti-diabetic properties. In addition, FFM inhibited the growth of all tested bacterial and fungal strains and showed the highest antibacterial activity against K pneumoniae strain. Future plans are needed to confirm these crucial results with in vivo experiments and to design suitable formulations for use in the pharmaceutical and food supplement industries.

In our study, mandrake root was studied and the appropriate experimental dose was determined in in vivo studies.

Arbia et al. (2019) in their research was carried out to evaluate the antioxidant degradation and enzyme inhibitory potential of two extracts (acetone and methanol) from two different parts (flower and leaf) of Mandragora autumnalis. It suggests that M autumnalis may be valuable as a natural agent for food and drug applications.

In our study, considering the potential of mandrake to be used as an anesthetic drug, the appropriate dose for daily use was investigated.

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Sarigül and Bekcan (2009) in their study on *D. magna*, LC50 values in 24- and 48-h static trials; 0.019 mg/L (95% confidence interval = 0.012 mg/L – 0.024 mg/L) over the 24-h period and 0.012 mg/L (95% confidence interval = 0.001 mg/L–0.016 mg/L) over the 48-h period detected.

In our study, it was calculated with the formula LC50 = (A + B) × 1/2, where A: the highest concentration at which no organisms die, B: the lowest concentration at which all organisms die. At 60-90 min, LC50 was found to be (30 + 90) × 1/2. Value of LC50 = 60.

Pino-Otín et al. (2019) found that the aqueous extract of the *A. absinthium* (wormwood) plant, which is used as a bionematoside, causes acute toxicity at low dilutions (up to 0.2% density) for non-target organisms and organisms according to their susceptibility levels. *D. magna* (LC50 ¼ % 0.236) > Vibriofisheri (LC50¼ % 1.85) > Chlamydomonas reinhardtii (LC50 ¼ % 16.49). Mandrake was found to be less lethal than *A. absinthium.* Yıldırım, in his study in 2015, observed Toxic Unit values after 24 h and 48 h for animal antibiotics Baytril-K and Entervet on *D. magna* were 61.25% and 224.5%, respectively, also observed for Clindane, Tetra, and Azro, which are antibiotics of human origin, as 776.7%, 115% and 300%, respectively. In this study, it was determined that the lethal effect of mandrake on *D. magna* was observed at rates above 50% concentration. In our study value of LC50 = 60.

Conclusion

The results obtained in this study showed that the consumption of mandrake extracts in the form of tea in the treatment of rheumatic pains by humans in Turkey carries a serious risk due to its toxic effect. This study will be an important reference for creating the necessary awareness. Hearing consumption among the public should be reviewed by taking into account the doses in the study.

The findings of this study can be used in the dose adjustment of the concentration ratios of these plant extracts in studies to be conducted on the use of mandrake plant as an anesthetic drug in fish.

According to the results obtained in our study, it has been determined that Mandragora plant has the potential to be used as an anesthetic drug in fish and studies to determine its anesthetic effect in fish can yield positive results.

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Conflict of interests. The authors declare that they have no conflict of interests in publishing this manuscript.

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