ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

The Effects of *Allium Czelghauricum* (Liliaceae), *Lathyrus Karsianus* (Fabaceae) and *Onosma Nigricaule* (Boraginaceae) Extracts on Oxidation Parameters in Malathion Treated Mice

Malatiyon Verilen Farelerde Oksidasyon Parametreleri Üzerine Allium Czelghauricum (Liliaceae), Lathyrus Karsianus (Fabaceae) ve Onosma Nigricaule (Boraginaceae)'den Elde Edilen Ekstraktların Etkileri

Dincer Erdag[1], Abdullah Dogan[2]

[1]Department of Medical Services and Techniques, Atatürk Vocational School of Health Services; [2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

ABSTRACT

Aim: *It was aimed to determine antioxidant properties of extracts obtained from Allium czelghauricum, Lathyrus karsianus and Onosma nigricaule species and antioxidant effects of plant extracts on malathion-induced oxidant parameters in mice.*

Material and Method: *Expermental animals were seperated into ten groups, each consisting of ten mice. First group was designed as a control group. Second group received 0.2 mL/kg of saline, and third group received the same dose of corn oil. Fourth (100 mg/kg malathion), fifth (100 mg/kg Allium czelghauricum), sixth (100 mg/kg Lathyrus karsianus), seventh (100 mg/kg Onosma nigricaule), eighth (100 mg/kg malathion+100 mg/kg Allium czelghauricum), ninth (100 mg/kg malathion+100 mg/kg Lathyrus karsianus) and the tenth group (100 mg/kg malathion+100 mg/kg Onosma nigricaule) were given daily intraperitoneally in the determined amounts. Applications were made for 21 days. After administration, total oxidant and total antioxidant capacities, body and liver weights, and histopathological changes in the liver were investigated in the serum and liver of mice.*

Results: *It is observed that plant extracts significantly inhibit the dose dependent concentrations of nitric oxide radical. A high amount of polyphenolic compounds were detected in plant extracts. While applied malathion mice weighed lighter, an increase in their liver was observed. Pathological changes were found in the liver of mice given malathion in histopalogical examinations. Total oxidant capacity (TOC) of serum and liver was significantly higher compared with control group; however, a decrease was observed in total antioxidant capacity (TAC). While the TOC in the serum and liver of mice given malathion and plant extract decreased compared to the malathion group, it was found that there was an increase in TAC.*

Conclusion: *In vitro study, it was revealed that methanol extracts of plants have antioxidant effects. In addition, it was determined that plant extracts exhibited antioxidant effects against the oxidant effect caused by malathion.*

Key words: *malathion; antioxidant; extract*

ÖZET

Amaç: *Allium czelghauricum, Lathyrus karsianus ve Onosma nigricaule türlerinden elde edilen ekstraktların antioksidan özellikleri ile farelerde malatiyon kaynaklı oksidan parametreler üzerine bitki ekstraktlarının antioksidan etkilerinin belirlenmesi amaçlanmıştır.*

Materyal ve Metot: *Deney hayvanları her grupta 10 adet fare olmak üzere toplam 10 gruba ayrıldı. Birinci grup kontrol grubu olarak tasarlandı. İkinci gruba 0,2 mL/kg dozda serum fizyolojik, üçüncü gruba ise yine aynı dozda mısır yağı verildi. Dördüncü (100 mg/kg malatiyon), beşinci (100 mg/kg Allium czelghauricum), altıncı (100 mg/kg Lathyrus karsianus), yedinci (100 mg/kg Onosma nigricaule), sekizinci (100 mg/kg malatiyon+100 mg/kg Allium czelghauricum), dokuzuncu (100 mg/kg malatiyon+100 mg/kg Lathyrus karsianus) ve onuncu gruptakilere (100 mg/kg malatiyon+100 mg/kg Onosma nigricaule) belirlenen miktarlardaki maddeler günlük olarak intraperitonal yolla verildi. Uygulamalar 21 gün süreyle yapıldı. Uygulamadan sonra farelerin serum ve karaciğerinde total oksidan (TOK) ve total antioksidan kapasite (TAK)'leri, vücut ve karaciğer ağırlıkları ile karaciğerde histopatolojik değişiklikler araştırıldı.*

Bulgular: *Bitki ekstraktlarının nitrik oksit radikalini doza bağlı olarak çalışılan konsantrasyonlarda istatistiksel yönden anlamlı şekilde inhibe ettiği görüldü. Bitki ekstraktlarının önemli miktarda polifenolik bileşikleri içerdiği tespit edildi. Malatiyon verilen farelerin vücut ağırlıklarında azalma meydana gelirken, karaciğer ağırlıklarında artış gözlendi. Histopalojik incelemelerde malatiyon verilen farelerin karaciğerinde patolojik değişimlere rastlandı. Malatiyon uygulanan farelerin serum ve karaciğerinde TOK düzeyi kontrol grubuna göre istatistiksel olarak önemli ölçüde artış gösterirken, TAK düzeyinde ise düşüş gözlenmiştir. Malatiyon ile birlikte bitki ekstraktı verilen farelerin serum ve karaciğerinde TOK düzeyi malatiyon grubuna göre düşüş gösterirken, TAK düzeyinin ise arttığı tespit edilmiştir.*

Sonuç: *İn vitro çalışmada bitkilerin metanol ekstraktlarının antioksidan özelliklerinin olduğu ortaya kondu. Ayrıca, malatiyonun neden olduğu oksidan etkiye karşı bitki ekstraktlarının antioksidan etki gösterdiği belirlendi.*

Anahtar kelimeler: *malation; antioksidan; özüt*

İletişim/Contact: *Dinçer Erdağ, Department of Medical Services and Techniques, Atatürk Vocational School of Health Services, Kafkas University, Kars, Turkey* • ***Tel:*** *0474 242 68 40* • ***E-mail:*** *dincererdag@hotmail.com* • ***Geliş/Received:*** *18.05.2021* • ***Kabul/Accepted:*** *19.12.2021*

ORCID: *Dinçer Erdağ, 0000-0001-7137-4403* • *Abdullah Doğan, 0000-0003-2008-6713*

Introduction

Malathion [O, O-dimethyl-S– (1.2-dicarbethoxyethyl) phosphorodithioate] is one of the organic phosphorous insecticides that have very prevalent usage against a variety of bugs for the protection of agricultural products and public health[1]. Nearly all chemical insecticides possess a neurotoxic effect, display toxic effects on the nervous system in target organisms[2]. Malathion is a kind of wide-spectrum insecticide, causes accumulation of acetylcholine on synapsis at nerve endings, inhibiting acetylcholinesterase, degrading acetylcholine that takes a role transmission of neural impulses in target organisms. Therefore, at target organisms that contact with the medication, firstly warnings emerge and later, paralysis follows these warnings[3].

Pesticides induce degradation of cellular activities, affecting cell structure and metabolism. One of the factors that lead to cellular damage is the formation of free radicals produced by pesticides[4]. These radicals occur continuously during metabolism in cells under aerobic conditions. The radicals formed under normal circumstances are removed from the environment by cell buffer systems. However, under certain conditions, excess free radicals arise and could not be disposed of by these buffer systems. If reactive oxygen types could not be eliminated with antioxidant defense systems, cellular damage emerges as a result of lipid peroxidation[5].

In many studies, it is illustrated that some plant varieties are good for diseases, other ones have features protecting from diseases. In the studies conducted on the plants with protective characteristics, it is determined that such plants carry substances that show a high-level antioxidant property. It is demonstrated in the studies carried out that such plants are rich in terms of chemical substances such as polyphenol, vitamins[6].

Plant varieties belonging to the Boraginaceae family may grow in Turkey. This family has 154 species and also a variety of around 2500[7]. Boraginaceae, a cosmopolite family show spreading mostly in tropical and mild regions, including Turkey[8].34 species and variety more than 300 belonging to this family grow in Turkey[9–11]. *Onosma nigricaule* about the Boraginaceae family is an endemic variety for Turkey. This plant variety finds an area of usage in the treatment of wounds and burns in the Eastern Anatolia Region[12]. The extracts obtained from some *Onosma* varieties, especially plant extract of *Onosma hispidum* is employed s antioxidant, antibacterial, antiviral, and anti-inflammatory agent in traditional medicine. In addition, *Onosma* varieties are also utilized in relieving pains and treatment the diseases like bronchitis, tonsillitis, hemorrhoid, etc. among the public. *Onosma hispida* is beneficial as a laxative and anthelmintic too. Furthermore, this plant is also administered against the ailments like inflammation, itch, wound, and kidney stone as well as the diseases such as eye diseases, blood disorders, and stomach aches[13].

Some plant species belonging to the Fabaceae family, which are also distributed in the flora of Turkey, have medical importance. It has been reported that it has traditionally been used in the treatment of various diseases[14]. Plant species belonging to this family are mainly consumed by humans and animals as foodstuffs, and some species are used in ornamental plants and pharmaceutical industries[15]. It has been reported that some species belonging to the family have antibacterial and antifungal activities[16], and some species also show strong antioxidant activity[17]. *Lathyrus* species are used worldwide as animal and human food. Pastor-Cavada et al.[18] researched the antioxidant activity of phenolic compounds found in the seeds of 15 wild *Lathyrus* species spreading in Southern Spain. It has been shown that the *Lathyrus* species studied have phenolic compounds with stronger antioxidant activity than the commonly consumed species such as soy, chickpea, and broad bean. *Lathyrus karsianus* is a plant species in the Fabaceae family and there are more than 200 species of the *Lathyrus* genus in the world[19].

Although the Liliaceae family is cosmopolitan, it is more common in tropical and temperate regions. In this family, there are important ornamental plants, aromatic plants, and vegetables as well as the plant species used in drug production[20]. The benefits of *Allium* species for human health are well known. For example; remarkably, *Allium sativum* (garlic) has prophylactic and therapeutic effects in some diseases (fungi). Evidence obtained from research has shown that the genus *Allium* plays an important role in the treatment and prevention of pathogenic infections, tumors, and cardiovascular diseases. Chemical compounds present in the *Allium* genus have been reported to potentially significantly reduce the level of lipid peroxidation in experimental animals[21]. One of the species of the genus *Allium*, *Allium czelghauricum* is in the Liliaceae family and is endemic to Turkey.

This study, it was aimed to determine the antioxidant effects of methanol extracts obtained from endemic plant species *Onosma nigricaule* (Boraginaceae),

Lathyrus karsianus (Fabaceae), and *Allium czelghauricum* (Liliaceae) against oxidation caused by malathion in mice (*Mus musculus*).

Material and Method

Plant material

Onosma nigricaule (Boraginaceae), *Lathyrus karsianus* (Fabaceae), and *Allium czelghauricum* (Liliaceae) plant species were collected from province Kars between May September 2011 and left for drying in the shade in a way not to expose to sun rays. After the drying procedure, only the foliage of the plants was received and ground through a grinder till becoming powder well, made ready for the procedure on taking the extract.

Animals

Approval was taken from Kafkas University Animal Experiments Local Ethics Committee for research (Decision no: 26.11.2010/48). A total of 100 mice (*Mus musculus*) of the same species who are not used in any study and not copulated before were used during the research. Male mice that have approximately 22–35 g weight, with weeks of 7–8, reached puberty period were taken the test. Mice were distributed in a manner to become 10 pieces in each group and placed in cages (Total 10 groups). Animals were accommodated in standard cages in an illuminated environment for 12 hours and in a dark environment for 12 hours, fed with normal mouse feed and tap water and as *ad libitum* in 22±2°C ambient temperature.

Preparation of plant extracts

Extract taken from plants was realized with the Soxhlet extraction system. A Jacketed heater was used for extract taking procedure. For extract taking process, methanol was used as a solvent. Samples of dried plant foliage were made powder via grinding. From this plant powder, 25 g was weighed and used for each test. It was placed in a roll made from filter paper. This roll was put in a soxhlet device. After that, a balloon incorporating 400 mL methanol was placed in a jacketed heater properly. The device was heated in a way methanol is boiled regularly (65 ^{0}C). This extract-taking procedure continued until the color of the solvent was clarified. After completion of the procedure, methanol was evaporated completely in an evaporator with water tromp under low pressure[22]. Extract substance was obtained in a tube. Later, extract substance found in the glass container was packaged in a manner to be protected from light with aluminum folia, stored in -35 ^{0}C. Total polyphenolic substance content of obtained extract, the radical-scavenging effect of nitric oxide (NO) was looked at. These effects were compared with standard substances known to have antioxidant capacity. These extracts were solubilized in physiological saline in the next stage later and injected into testing animals intraperitoneally.

Establishing experiment groups

The following test groups were formed in a manner that 10 male mice exist in each group. The chemical substance and extracts were solubilized with proper solutions in a way chemical substance and extracts are 100 mg/10 mL (Malathion was solubilized in corn oil, plant extracts in physiological saline). In conclusion, excluding the negative control group, solvent, physiological saline, or corn oil were injected with the calculation of 0.2 mL/20 g mouse to other groups. 1st Group: This group was designed as a negative group, no substance application was made to mice (C). 2nd Group: Physiological saline (0.9% NaCl), carrier substance of plants extract was applied to mice daily intraperitoneally (ip) during 21 days (0.2 mL/20 g) (S). 3rd Group: Corn oil, carrier substance of malathion was applied to mice daily ip (0.2 mL/20 g) (CO). 4th Group: Malathion was solubilized in corn oil for 21 days and applied to mice ip at daily 100 mg/kg dose (0.2 mL/20 g) (M). 5th Group: Plant extract *Allium czelghauricum* (Liliaceae) was solubilized in serum physiologic (0.9% NaCl) for 21 days and applied to mice ip at daily 100 mg/kg dose (0.2 mL/20 g) (A). 6th Group: Plant extract *Lathyrus karsianus* (Fabaceae) was solubilized in serum physiologic (0.9% NaCl) for 21 days and applied to mice ip at daily 100 mg/kg dose (0.2 mL/20 g) (L). 7th Group: Plant extract *Onosma nigricaule* (Boraginaceae) was solubilized in serum physiologic (0.9% NaCl) for 21 days and applied to mice ip at daily 100 mg/kg dose (0.2 mL/20 g) (O). 8th Group: Plant extract *Allium czelghauricum* (Liliaceae) at 100 mg/kg dose + malathion at 100 mg/kg dose was applied daily to mice ip for 21 days (0.2 mL/20 g) (MA). 9th Group: Plant extract *Lathyrus karsianus* (Fabaceae) at 100 mg/kg dose + malathion at 100 mg/kg dose was applied daily to mice ip for 21 days (0.2 mL/20 g) (ML). 10th Group: Plant extract *Onosma nigricaule* (Boraginaceae) at 100 mg/kg dose + malathion at 100 mg/kg dose was applied daily to mice ip for 21 days (0.2 mL/20 g) (MO).

Determination of total polyphenolic substance

Determination of total soluble phenolic substances of the extracts of plant *Onosma nigricaule, Lathyrus karsianus*, and *Allium czelghauricum* prepared in methanol was established by using Folin-Ciocalteu separator according to Slinkard and Singleton method[23].

Nitric oxide radical scavenging activity

Nitric oxide free radical scavenging activity of the methanol extracts obtained from plant *Onosma nigricaule, Lathyrus karsianus*, and *Allium czelghauricum* was measured with partial modification of Badami et al.[24] and Kumar et al.[25] methods. Sodium nitroprusside produces NO by itself in aqueous solutions and physiologic pH and this NO radical also generates nitrite (NO_2) ions, interacting with the oxygen in the environment. Nitrite anion formed was colored with Griess reaction at 548 nm, NO determination in the environment was made by reading at spectrophotometry[26].

Determination of total antioxidant and total oxidant capacities

Antioxidant and quantity of antioxidant in plasma and liver tissue was measured employing the use of total antioxidant capacity (TAC) assay kit and total oxidant capacity (TOC) assay kit (Rel Assay Diagnostics, Clinical Chemistry Solutions) [27]. Concerning antioxidant and oxidant capacities in plasma and liver tissue, the analysis method was used by modifying partially.

Measuring live weight

Just before administering medication, the weights of animals in all groups were measured (the first day that application is started). Animals were put in a container whose tare is determined in a way to obstruct their move and weighted in a digital scale. This procedure was repeated every week during the application period. After the application ended (21st day), animals were weighed again before euthanasia. Live weights of animals were compared, using statistical methods and the difference between groups was detected.

Measuring the weight of the liver

At the end of the study, the liver tissues which are received from testing animals killed with cervical dislocation under ether anesthesia were put in a sterile container and weighted in sensitive digital scales. Weights of liver tissues taken from animals were compared with the use of statistical methods and difference between groups was determined.

Histopathological examinations

At the end of the study, liver tissues of the experimental animals that were euthanized were taken and examined histopathologically. Liver samples taken from all experimental mice were detected in 10% formaldehydesolution. After the tissues were serially passed through graded alcohols, methylbenzoate, and benzol solutions, these tissues were blocked in paraffin. Sections of 5 μm thickness were taken from the tissues in the paraffin blocks and stained with Hematoxylin-Eosin (HE)[28] and evaluated under a light microscope (Olympus BX51; Olympus Optical Co., Osaka, Japan). Microscopic pictures were taken from the cases deemed necessary. In the evaluation of liver damage, the liver surface area observed in experimental and control groups; (i) extent of hepatocellular necrosis, (ii) cloudy bloating and hydropic degeneration, (iii) vacuolar degeneration, (iv) bile duct hyperplasia, (v) anisocytosis and anisocytosis, (vi) severity and prevalence of polymorph and mononuclear inflammatory cell infiltration were evaluated semi-quantitatively in six different categories.

Statistical calculations

One-Way ANOVA test was utilized for statistical calculations. Test groups were studied comparatively with control groups. Results were determined in average ± standard deviation (X ± SD) and showed a statistical difference of $p<0.05$. All calculations were utilized, using SPSS (16.0–2010) packaged software.

Results

Determination of total polyphenolic substance

Polyphenols are compounds that catch radicals (thanks to hydroxyl groups). They react with free radicals and turn them into ineffective, so harmless compounds. These effects may also be called free radical scavenging (cleaning) activity. Furthermore, these features gain antioxidant characteristics to polyphenolic compounds. Therefore, their levels in plants are very important. As they are efficient on antioxidant parameters, levels of phenolic compounds in studied plants were determined in the research. The amount of the phenolic substance found in plants was detected, using Folin-Ciocalteu solution. Total phenolic substance content of methanol extracts of *Onosma nigricaule, Lathyrus karsianus*, and *Allium czelghauricum* plants was. For this calculation, it was benefited from the standard pyrocatechol graphic presented below and

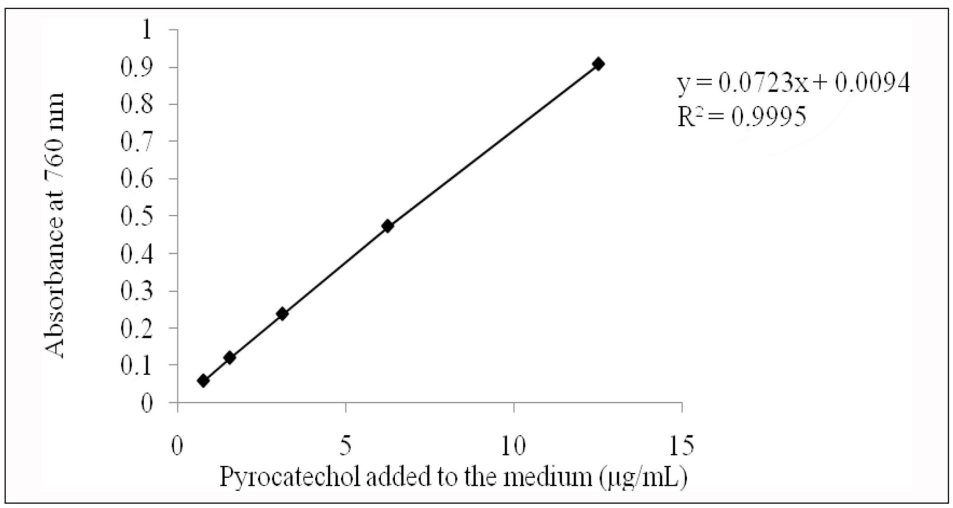
the following formula. Results were determined as phenolic substance equal to microgram pyrocatechol. From standards, it was calculated that the curve obtained is r^2=0.9995 (Graph 1).

Pyrocatechol(µg)=[Absorbance 0.0094]/0.0723(r^2=0.9995)

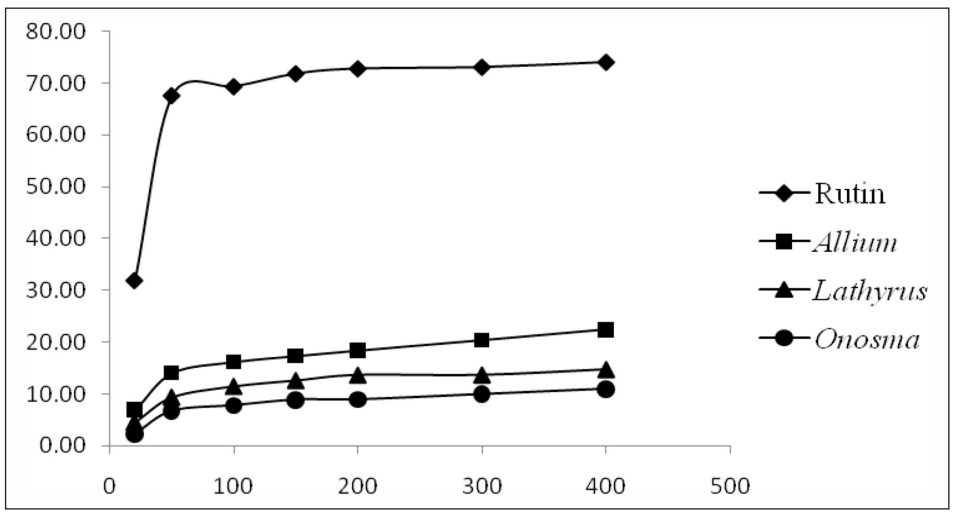
From here, it was detected that 1 mg of *Onosma nigricaule* contains 43.53 µg, 1 mg *of Lathyrus karsianus* 54.15 µg, and 1 mg of *Allium czelghauricum* contains 62.85 µg conjugate substance.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging % activities of *Onosma nigricaule, Lathyrus karsianus*, and *Allium czelghauricum* and routine standard substance are shown graphically, taking averages of data obtained after three testings (Graph 2). It was stated that nitric oxide scavenging effects of the plants show parallelism with the polyphenolic compound they contain. It was determined that the nitric oxide scavenging effect decreased following the order of *Allium czelghauricum, Lathyrus karsianus*, and *Onosma nigricaule* plant extract (routine 72% at 400 µg/mL dose, *Allium czelghauricum* extract 22.44%, *Lathyrus karsianus* extract 14.70% and *Onosma nigricaule* extract cleared 10.96% NO). The difference was found significant between routine and plant extracts (P<0.01). Considering the rate of a polyphenolic compound, it was detected that plant extracts had the scavenging effect at adegree close to that of a routine substance.



Graph 1. *Pyrocatekol standard graphic.*



Graph 2. *Comparison of the NO radical scavenging activities of Allium czelghauricum, Lathyrus karsianus, Onosma nigricaule, and rutin.*

TAC of plasma

Early death was observed in the groups and was not included in the calculation. The changes observed in the plasma TAC levels of the groups are shown in Table 1. When plasma TAC was compared as per groups, TAC groups of control, serum, and corn oil groups were found higher than other groups. TAC was detected low in the group that malathion was given. TAC degrees calculated in the groups that only plant extract was given illustrated similarity with control groups. However, according to the results obtained, it was observed that TAC value increased due to giving plant extract with malathion. The difference between plasma TAC levels attained from the plant extract groups that were given along with malathion and the malathion with a control group was found statistically significant. Concerning all groups, it was seen that TAC levels were low about control and the groups that serum and corn oil were given.

TOC of plasma

The changes determined in the plasma TOC of the groups are shown in Table 1. Plasma TOC was found low for control, serum, and corn oil. It was witnessed that the TOC level was at the highest level for the malathion group. It was detected that TOC levels for the groups that plant extract was administered are close to the controlling group. According to the results obtained, it was determined that plant extract decreases the oxidation malathion that emerges. However, the results taken from the group that malathion and plant extract were given together were detected higher than that of the control group. The difference is the highest between malathion and control group, was found statistically significant ($p<0.05$). TOC levels did not show a substantial difference statistically among the groups that malathion and vegetable extract were administered together.

TAC of liver

The changes determined in the liver TAC of the groups are shown in Table 2. It was observed that TAC in the liver lowered for the group that malathion was given. It was detected that plant extract increased TAC for the groups that malathion was administered. This difference was found significant than control groups.

TOC of liver

Variations in liver total oxidant capacities of groups are illustrated in Table 2. TOC of liver were found higher than the group malathion was given. As per the results, it was detected that compared to the control group, plant extract decreased the oxidation caused by malathion ($p<0.05$). Between the groups of control, serum, control oil, and the group only plant extract were administered, no apparent difference was observed from the point of liver antioxidants.

Measuring body weight

The changes in the body weights of the experimental groups on day 0th and day 21st are shown in Table 3. When the live weights of groups were compared in a slice of time specified, it was determined no substantial difference was available between groups on the 0th day ($P>0.05$). However, it was monitored that a decrease was present for body weights of 21st day for malathion group as compared to other groups. This was also found statistically significant ($P<0.05$).

Measuring liver weight

The changes determined in the liver weights of the experimental groups are shown in Table 4. When liver weights of testing groups were checked against daily test groups, it was seen that an increase occurred in the malathion group compared to other groups. This was also found statistically significant ($P<0.05$).

Histopathological findings

Damage to liver samples taken from mice in all groups was graded semi-quantitatively according to the criteria reported in the material and method. In the control group, diffuse, focal parenchymal, or centrilobular (periacinar, zone 3) liver necrosis, which is the most important criterion of liver intoxication, could not be detected (Fig. 1). Parenchymal, mild-moderate, coagulative necrosis was detected in the liver of mice in the malathion group. In addition, vacuolar degeneration in hepatocytes around the vena centralis and multinucleated hepatocytes regenerating in the liver parenchyma were observed (Fig. 2). A limited anisocytosis and anisocytosis were observed in the liver of mice in the group's given plant extract with malathion (Fig. 3).

Discussion

Today, many pesticide species used for agricultural pest control exist. These are obtained naturally and semi-synthetically. The synthetic compounds may be classified as compounds with organic chlorine, phosphorus, carbamate, pyrethroid, and nicotinamide. One of the

Table 1. *Observed changes in plasma total antioxidant capacity and total oxidant capacity*

Groups	TAC (mmolTroloxEquiv. /L)	TOC (μmol H_2O_2Equiv. /L)
C (n=7)	1.047±0.053[a]	0.157±0.636[a]
S (n=7)	1.069±0.043[a]	0.178±1.244[a]
CO (n=7)	1.091±0.072[a]	0.181±0.823[a]
M (n=10)	0.421±0.029[b]	1.078±2.166[b]
A (n=10)	0.956±0.092[ac]	0.241±1.026[ac]
L (n=10)	0.921±0.060[ac]	0.257±0.482[ac]
O (n=9)	0.879±0.097[ac]	0.262±0.546[ac]
MA (n=9)	0.732±0.030[d]	0.694±1.039[d]
ML (n=9)	0.745±0.078[d]	0.705±0.702[d]
MO (n=9)	0.670±0.034[d]	0.759±0.634[d]

[a,b,c,d]: The difference between the means with different letters in the same column is statistically significant (P<0.05). (TAC: Total antioxidant capacity, TOC: Total oxidant capacity, C: Control, S: Physiological saline, CO: Corn oil, M: Malathion, A: *Allium czelghauricum*, L: *Lathyrus karsianus*, O: *Onosma nigricaule*, MA: Malathion + *Allium czelghauricum*, ML: Malathion + *Lathyrus karsianus*, MO: Malathion + *Onosma nigricaule*).

Table 2. *Changes determined in liver total antioxidant capacity and total oxidant capacity*

Groups	TAC (mmolTroloxEquiv. /L)	TOC (μmol H_2O_2Equiv. /L)
C (n=7)	2.112±0.183[a]	19.854±0.669[a]
S (n=7)	2.178±0.185[a]	18.906±0.935[a]
CO (n=7)	2.158±0.186[a]	19.597±0.410[a]
M (n=10)	1.441±0.160[b]	28.092±0.631[b]
A (n=10)	1.956±0.163[ac]	20.203±0.661[a]
L (n=10)	1.945±0.167[ac]	21.254±0.632[a]
O (n=9)	1.928±0.140[ac]	20.883±0.569[a]
MA (n=9)	1.618±0.182[d]	23.678±1.284[c]
ML (n=9)	1.588±0.091[d]	23.924±1.101[c]
MO (n=9)	1.569±0.120[d]	24.505±0.854[c]

[a,b,c,d]: The difference between the means with different letters in the same column is statistically significant (P<0.05). (TAC: Total antioxidant capacity, TOC: Total oxidant capacity, C: Control, S: Physiological saline, CO: Corn oil, M: Malathion, A: *Allium czelghauricum*, L: *Lathyrus karsianus*, O: *Onosma nigricaule*, MA: Malathion + *Allium czelghauricum*, ML: Malathion + *Lathyrus karsianus*, MO: Malathion + *Onosma nigricaule*).

Table 3. *Body weight measurement (g)*

Groups	0. Day (X ± Sx)	21. Day (X ± Sx)
C (n=7)	27.86±2.77	31.86±3.01
S (n=7)	27.86±2.80	31.00±2.45
CO (n=7)	27.86±2.42	30.57±1.62
M (n=10)	27.27±2.31	26.72±1.33*
A (n=10)	27.90±2.63	32.20±1.23
L (n=10)	26.80±2.87	32.50±1.35
O (n=9)	27.56±2.08	32.22±1.15
MA (n=9)	27.22±1.58	30.78±1.41
ML (n=9)	27.78±1.58	30.56±1.17
MO (n=9)	28.00±3.43	30.67±2.52
General average	27.58±0.76	30.90±0.56

*: Statistically significant (P<0.05). (TAC: Total antioxidant capacity, TOC: Total oxidant capacity, C: Control, S: Physiological saline, CO: Corn oil, M: Malathion, A: *Allium czelghauricum*, L: *Lathyrus karsianus*, O: *Onosma nigricaule*, MA: Malathion + *Allium czelghauricum*, ML: Malathion + *Lathyrus karsianus*, MO: Malathion + *Onosma nigricaule*).

Table 4. *Liver weight measurement (g)*

Groups	X ± Sx
C (n=7)	1.92±0.15
S (n=7)	1.84±0.13
CO (n=7)	1.78±0.12
M (n=10)	2.48±0.10*
A (n=10)	1.65±0.13
L (n=10)	1.91±0.05
O (n=9)	1.90±0.06
MA (n=9)	1.99±0.09
ML (n=9)	1.96±0.09
MO (n=9)	1.98±0.06
General average	1.87±0.03

*: Statistically significant (P<0.05). (TAC: Total antioxidant capacity, TOC: Total oxidant capacity, C: Control, S: Physiological saline, CO: Corn oil, M: Malathion, A: *Allium czelghauricum*, L: *Lathyrus karsianus*, O: *Onosma nigricaule*, MA: Malathion + *Allium czelghauricum*, ML: Malathion + *Lathyrus karsianus*, MO: Malathion + *Onosma nigricaule*).

well-known groups among these is comprised of insecticides with organic phosphor. Within organic phosphorous insecticides, many compounds like malathion, parathion, dichlorvos, etc. are available. Malathion is among the most widely used organic phosphorus. They are absorbed easily from mucosas and transmitted into the blood. It exposes to bio-activation in the body. Their effect mechanism is associated with autonomous ganglions and the enzyme degrading acetylcholine, acting as transmitter substance at neuromuscular junctions of the parasympathetic nervous system, in other words, inhibiting acetylcholinesterase irrevocably. However, it is also established that malathion enhances free radical formation. Determination of the roles of free radicals in aging, the emergence of diseases like tumors reveals how important chronic malathion exposure is.

In this study, the effects of methanol extract obtained from plant species *Allium czelghauricum*, *Lathyrus karsianus* ve *Onosma nigricaule* on oxidative stress induced by malathion were investigated. The research was planned in vitro and *in vivo*. *In vitro* study, levels of polyphenolic compounds in plants were detected. Later, methanol extracts of the plant were applied on

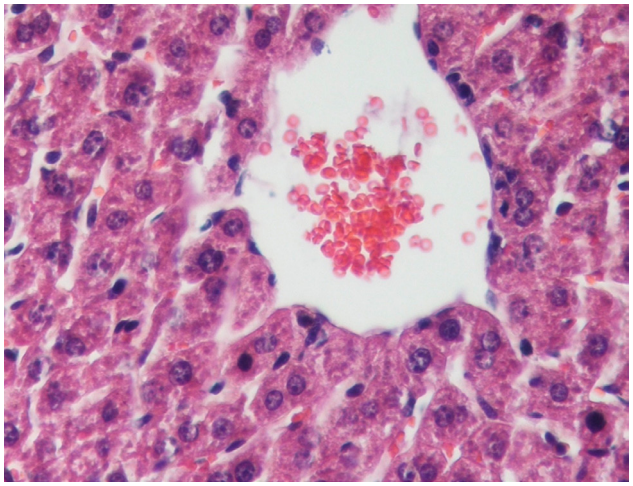


Figure 1. *Relatively normal hepatocytes around vena centralis. (HE x40).*

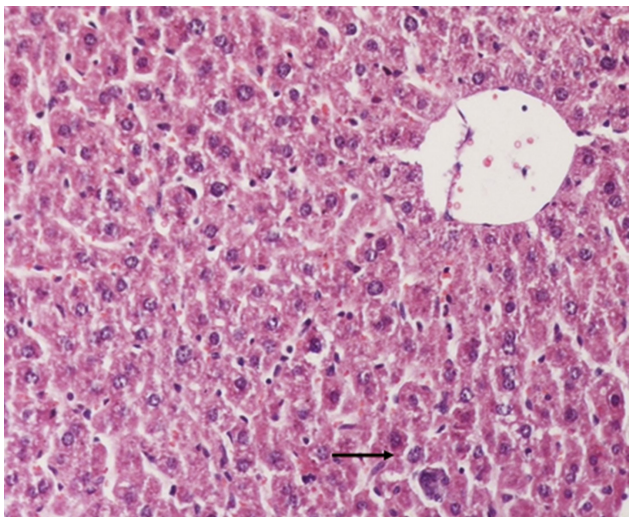


Figure 2. *Vacuolar degeneration of hepatocytes around vena centralis and multinucleated hepatocyte regenerated in liver parenchyma (arrow). (HE x20).*

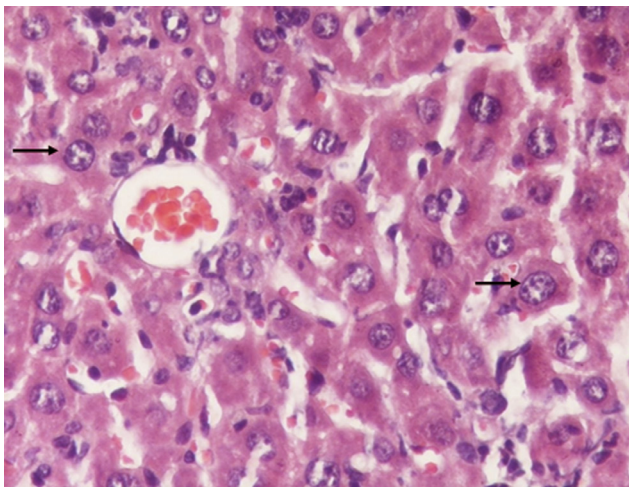


Figure 3. *Mild anisocytosis and anisokaryosis in hepatocytes (arrows). (HE x40).*

rats and its effects on oxidative parameters caused by malathion were determined. In general, it was observed that plant extracts used in the research scavenge NO radical formed experimentally. When these effects were matched against standard antioxidant substances, it was shown that plant extracts had quite high antioxidant activity. Furthermore, it was also detected that these antioxidant effects increased depending on higher concentrations of plant extracts. The plants studied are endemic, they grow in the region. Lack of any former study related to this kind of plant put forward the importance of the study. Additionally, in terms of understanding the importance of endemic plant species and protecting these plants, research results are evaluated with great importance and as attention-grabbing.

Carrying medication raw materials by plants and understanding their importance in treatment at the same time has caused making prevalent studies on them in recent years. At the same time, leading to serious disease of free radicals has induced concentration of studies on antioxidant impacts of plants. It is seen many studies are carried out to that end. The Liliaceae family includes a large and significant portion of flowering plants, and besides the plant species used in drug production in this family, there are important ornamental plants, aromatic plants, and vegetables[29]. The benefits of *Allium* species for human health are well known. It has been confirmed as a result of many studies that some precursor compounds and sulfur-containing compounds of *Allium* species have anti-oxidative activity. These chemical compounds have been reported to potentially significantly reduce the level of lipid peroxidation in experimental animals[30]. In this study, polyphenolic compounds among antioxidant substances were found higher in *Allium* species than in other plants (62.85 μg/mg). Tepe et al.[31] researched the antioxidant activities of methanol extract of a total of 5 *Allium* species, two of which are endemic, in the flora of Turkey. 1.1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities of extracts obtained from plants were investigated, and also carotene/linoleic acid levels were analyzed. As a result, it has been reported that these *Allium* species, which are widely distributed in the flora of Turkey, have potent antioxidant properties. Ahmed et al.[32] have analyzed the impact of ginger (*Zingiber officinale* Rosc) on the oxidative stress formed by malathion for rats. They have evaluated lipid peroxidation, glutathione, and dependent enzymes and the enzymes scavenging free oxygen radicals to the

rats exposed to malathion sub-chronically. They have determined the existence of an increase in malondialdehyde (MDA) level in serums, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) enzyme activities in erythrocytes, glutathione reductase (GR) and glutathione-S-transferase (GST) activities of the rats that malathion is administered at 20 ppm level for four weeks. Furthermore, a drop is observed in glutathione (GSH) levels in the blood. It is monitored that ginger (*Zingiber officinale* Rosc) given with feed to rats daily decrease lipid peroxidation and oxidative stress considerably. Finally, they have detected that free radical formation increases at organophosphate-induced poisoning, ginger has a protective effect against that. Therefore, it is indicated that ginger is a plant having medical importance and the products generated from this plant may be beneficial too.

Many studies have been conducted on herbal polyphenolic compounds, and it has been reported that these compounds have very strong antioxidant activity, neutralize free radicals in the body, prevent cardiovascular diseases, and even delay aging[33]. Some plant species belonging to the Fabaceae family have medical importance. It has been reported that it has traditionally been used in the treatment of various diseases[34]. It has been reported that some species belonging to the family have antibacterial and antifungal activities[35], and some species also show strong antioxidant activity[36]. Pastor-Cavada et al.[18] researched the antioxidant activity of phenolic compounds found in the seeds of 15 wild *Lathyrus* species spreading in Southern Spain. It has been shown that the *Lathyrus* species studied have phenolic compounds with stronger antioxidant activity than the commonly consumed species such as soy, chickpea, and broad bean.

Pesticides cause significant changes in antioxidative enzyme metabolism both in natural plant species and in cultivated ecological species. Acute or chronic toxic effects include mutagenicity and organ toxicity. Alp et al.[37] investigated the effects of caffeic acid phenyl ester and ellagic acid on oxidative stress caused by acute malathion toxicity in rats. They have shown that caffeic acid phenyl ester and ellagic acid have antioxidant effects on oxidative stress in acute malathion intoxication. Cadirci et al.[38] has searched the effect of the root extract obtained from plant *Onosma armeniacum* on the oxidative stress formed on ethanol in stomach tissue of rats. The same researchers have examined some oxidant and antioxidant parameters in stomach tissue. They have detected the vegetable extract at 25, 50, 100, and 200 mg/kg doses, used in the study decreases ethanol-based stomach ulcer substantially, additionally hinders the decrease in total glutathione level depending on the damage occurred in stomach tissue of the rats that ethanol is applied. The roots of *Onosma* species are used for the treatment of various diseases such as bronchitis, tonsillitis, hemorrhoid, and relieving pains. Tosun et al.[39] have investigated anti-inflammation and antinociceptive activities of the chloroform and ethanol extract acquired from *Onosma aucheranum*, *Onosma isauricum*, *Onosma sericeum*, *Onosma tauricum* Pallas ex Willd. var. *Brevifolium* and *Onosma tauricum* Pallas ex Willd. var. *Tauricum* species that show spreading in Turkey. In conclusion, *Onosma aucheranum*, *Onosma isauricum, and Onosma sericeum* species have displayed efficient anti-inflammation and antinociceptive activity.

Consequently, the data obtained with tests that are conducted with various free radical scavenging systems in *vitro* test environments about methanol extract taken from the foliage of the plant *Allium czelghauricum*, *Lathyrus karsianus,* and *Onosma nigricaule* have revealed these plants free radical scavenging and antioxidant activity meaningfully. This circumstance may result from the phenolic compounds present within the plant.

The effect of plant extracts that in vitro antioxidant impact is determined on antioxidant and oxidant parameters for the rats that malathion is administered in vivo test environment have been investigated. Malathion has given rise to an increase in total oxidant capacity of liver tissue and plasma for rats and accordingly it is detected that it also enhances oxidative stress. It is considered that this increase depends on the escalation in the amount of free radical. At the same time, malathion has caused a decrease in the body weights of test animals. Again, an increase is observed for liver weights of rats that malathion is administered. Organophosphate insecticides have been shown to cause a decrease in the body weight of experimental animals in studies[40,41,42]. In this study, a decrease in body weight and body weight gain of the experimental animals was observed. 3 weeks after the application, compared to the group treated with malathion and plant extracts, compared to the control groups. It is estimated that the reason for this decrease in body weight may arise due to nutrient intake and poisoning. Because it was observed that the food intake for 3 weeks decreased significantly in the experimental animals in

these groups compared to the control groups. However, necrosis and atrophic structures in tissues can also cause a decrease in body weight.

In addition, pesticides are known to cause histopathological and cytopathological changes in various tissues of mammals as well as other living species. The liver and kidneys are among the tissues most damaged by pesticides. Because pesticides are metabolized in the liver and are generally excreted by the kidneys[41,43–45]. In addition to the direct interaction of pesticides with cell structures, it is thought that toxic intermediates resulting from changes in metabolism also play a role in the emergence of these negative effects. Kalender et al.[46] examined the effect of vitamin C and E on malathion-induced hepatotoxicity in rats. The rats were given daily 27 mg/kg malathion and 200 mg/kg vitamin C + vitamin E by oral gavage for four weeks. The liver tissues of both the malathion-given group and the malathion + vitamin given group were examined histopathologically by light microscopy analysis, and some pathological disorders were found in the rats in the malathion group. As a result, it has been stated that vitamins C and E can reduce the hepatotoxicity caused by malathion, but their protective effects are limited. In our study, the liver tissues of both the malathion group and the groups that were given malathion and plant extract were examined histopathologically by light microscopy analysis. Some pathological disorders were observed in the mice in the group given malathion. Plant extracts, on the other hand, reduced these pathological disorders. This may arise from the oxidative stress caused by malathion. Plant extracts have also lowered this oxidative stress. It is monitored an increase in total antioxidant capacities of liver tissues and plasma of the testing animals plant extracts are along with together malathion. This case means that plant extracts show an anti-oxidative effect against the oxidative impact free radicals induce. Therefore, it is concluded that conducting a variety of studies in pharmacognosy, pharmacodynamic, pharmacokinetic, and pharmaceutical chemistry fields is required for a better understanding of the medical importance of tested plants.

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