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# The Use of Macerated Garlic (*Allium Sativum L.*) Oil in Preventing the Postoperative Adhesions in Rats

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Garlic, Histopathology, Intraabdominal, Olive Oil, Surgical Adhesions

**Abstract:** In this study, the efficiency in preventing the postoperative adhesions by intra-abdominal use of the macerated oil obtained from Taşköprü Garlic (Kastamonu/Türkiye) was investigated. In the study, the macerated oil prepared using fresh garlic in olive oil at a concentration of 1.2 g/ml was used. Chemical structure of the macerated oil was analyzed by FTIR and GC-MS. Within the scope of in vivo experiments, cecal abrasion were applied to Wistar Albino rats (n=8) under general anesthesia. The physiological saline solution in control group (K) and different doses of macerated oil (0.5-1ml) in study groups (S1-S2) were dropped on the abrasion-zone. On postoperative days 3-28, the adhesions were evaluated during macroscopic observations and classified according to macroscopic adhesion score (MAS) between 0-4. At this stage, tissue samples were taken for histopathological examination from the subjects with adhesions and histopathological adhesion scoring (HAS) was performed between 0-3. The intra abdominal-adhesion was detected in all the subjects on postoperative day 3. On postoperative day 28, adhesions were observed at the MAS-2 level in K subjects, while there was no adhesion in S1-S2 subjects. In addition, histopathological adhesion was determined at the level of HAS-1 in all cases on day 3 and at the HAS-2 level in the K subjects on day 28. In conclusion, the garlic macerated oil, obtained by soaking fresh garlic in olive oil for a while, is an effective anti-adhesion agent which will be easily produced and applied by clinicians at low cost.



# Sıçanlarda Postoperatif Adezyonların Önlenmesinde Maserat Sarımsak (*Allium Sativum L.*) Yağı Kullanımı

**Anahtar Kelimeler**
Cerrahi Adezyonlar, Histopatoloji, İntra-abdominal, Sarımsak, Zeytinyağı

**Öz:** Bu çalışmada Taşköprü Sarımsağı'ndan (Kastamonu/Türkiye) elde edilen maserat yağın intra-abdominal kullanımının, ameliyat sonrası yapışıklıkları önlemedeki etkinliği araştırıldı. Çalışmada, zeytinyağında 1.2 g/ml konsantrasyonda taze sarımsakla hazırlanan maserat yağı kullanıldı. Maserat yağın kimyasal yapısı FTIR ve GC-MS yöntemleriyle analiz edildi. İn vivo deneyler kapsamında Wistar Albino cinsi sıçanlara (n=8) genel anestezi altında sekum abrazyonu uygulandı. Kontrol grubunda (K) serum fizyolojik solüsyonu ve çalışma gruplarında (S1-S2) farklı dozlarda (0,5-1ml) sarımsak maserat yağı abrazyon bölgesine damlatıldı. Postoperatif 3. ve 28. günlerde, makroskobik gözlemler sırasında adezyonlar değerlendirildi ve 0-4 arasında makroskobik adezyon skoruna (MAS) göre sınıflandırıldı. Bu aşamada adezyonlu olgulardan histopatolojik inceleme için doku örneği alındı ve 0-3 arasında histopatolojik adezyon skorlaması (HAS) yapıldı. Postoperatif 3. günde, tüm deneklerde intra-abdominal adezyon tespit edildi. Postoperatif 28. günde K deneklerinde MAS-2 seviyesinde adezyonlar görülürken, S1-S2 deneklerinde adezyon olmadığı belirlendi. Ayrıca 3. günde tüm olgularda HAS-1 düzeyinde ve 28. günde K deneklerinde HAS-2 düzeyinde histopatolojik adezyon belirlendi. Sonuç olarak, taze sarımsağın bir süre zeytinyağında bekletilmesiyle elde edilen sarımsak maserat yağı klinisyen hekimler tarafından kolaylıkla üretilip uygulanabilecek, düşük maliyetli ve etkili bir adezyon önleyici ajandır.

## 1. INTRODUCTION

Intra-abdominal adhesion is a pathological condition, which is observed after various surgical interventions, has a high incidence [1-5], and can cause various complications including stomachache, intestinal obstruction, and female infertility [5, 6]. The incidence rate of postoperative adhesions, which are known to occurs on the 3rd and 5th postoperative days, after abdominal surgery varies between 55 and 94 % [1]. In a prospective study examining 1000 patients who have undergone laparoscopic surgery at least once, adhesion incidence was reported to be 21.1% (n=211) and it was emphasized that the adhesions found in intestines in 28% (n=59) of cases [2]. Various agents and methods were analyzed regarding the prevention of adhesion and/or reducing its incidence [1, 3, 4, 6-9]. In a study using commercial garlic oil, authors reported no positive result [4], whereas several studies showed that garlic oil was successful in preventing adhesion at certain rates [3, 7].

Garlic is a valuable raw material that is included in many pharmacopeias such as African, European, and American pharmacopeia, and used in foods, medications, and dermo-cosmetics [10]. Garlic have been used since the ancient ages [7, 11, 12], the antibacterial effect of garlic oil was proven by Louis Pasteur in the 19th century [11, 13] and, stating that it was as effective as penicillin [11], garlic oil could be used for medical purposes [11, 13]. The antiseptic properties of garlic originates from allicin (di-allyl-thiosulfate), one of the sulfurous essentail oil [11]. Thanks to its organosulfur components [di-allyl sulfide, di-allyl disulfide (DADS), di-allyl trisulfide (DATS), and di-allyl tetrasulfide], garlic was reported to be protective against various diseases [3, 4, 7, 10, 11, 13] and to have hypolipidemic, antihypertensive, antioxidant [10-14], anticarcinogenic [12-15], antimicrobial, antithrombotic, fibrinolytic, and wound-healing effects [3, 4, 7, 10, 11, 13]. It is recommended to use essential oils obtained from fresh garlic as anticarcinogenic and their aqueous solutions as herbal medication [15]. The medicinal importance of garlic emerging as a result of its use as a therapeutic and protective instrument against diseases is much higher than its value as a food [11].

Being one of the most important agricultural products of Kastamonu, “Taşköprü Garlic” was registered with geographical indication by the Turkish Patent and Trademark Office in 2010 [16] and European Union in 2021 [17]. Rich selenium content in agricultural lands of Taşköprü district [10] ensures high selenium content in the composition of Taşköprü Garlic and it is the richest garlic variety grown in Turkey in terms of sulfurous essential oils and their derivatives [11]. Taşköprü Garlic is the richest garlic variety grown in Turkey in terms of sulfurous essential oils and their derivatives [11].

Maceration is a method that is used for extracting essential oils and active compounds from herbs. Offering an extraction time longer than the modern approaches do, maceration is preferred for its lower cost [12, 18]. In macerated oils obtained by keeping garlic cloves in herbal oils, alliin rapidly converts to allicin which is the main thiosulfate in garlic degrading into lipophilic products such as dithiins and ajoen [12, 14]. It was reported that the use of macerated garlic extract products might contribute to wound healing [15].

Considering the bioactive characteristics of garlic (*Allium sativum L.)* accepted as a natural and effective therapeutic agent [12, 13], it was thought that the macerated lipogenic extract to be obtained by macerating it in olive oil [12, 14] having a high level of bioactive properties might be effective in preventing postoperative intra-abdominal adhesion formation, which causes many complications and courses with high morbidity/mortality in veterinary and human medicine. In the present study, the effectiveness of the intra-abdominal use of macerated oil of garlic prepared with olive oil in preventing postoperative adhesions were investigated.

## 2. MATERIAL AND METHOD

First, the macerated garlic oils were prepared in the study. After chemical analyses were done, the most suitable one was determined. Then, macerated garlic oils were applied to the experimental group subjects at different doses, whereas control group subjects were given routine practice with physiological saline solution. The data were rated using macroscopic and histopathological adhesion scoring and comparatively discussed.

### 2.1. Ethical Statement

Upon the ethics committee approval [date of 13.05.2019 (26.02.2021-altered) and number of 2019/23 (2021/10-altered)] from the Animal Experiments Local Ethics Committee of Kastamonu University. The present study was carried out in the Experimental Animals Unit in Kastamonu University’s Faculty of Engineering and Architecture.

### 2.2. Experimental Groups

In the in vivo experiments, a total of 8 (6 female and 2 male), Wistar Albino rats (*Rattus albus*), aged 8-12 weeks and 200-300 g of live weight were included in the study. Rats were housed in conventional cages in pairs under controlled standard laboratory conditions (12 h dark/12 h daylight, 45%-55% of humidity rate, and room temperature at 20-22°C) and were fed ad libitum on filtered tap water and pellet feed approved by Turkish Standards Institute. Subjects were divided into a control group (Group K) (n=4) and an experimental group (n=4). The rats in the experimental group were divided into two subgroups according to received treatmens as 0.5 ml (Group S1) and 1 ml (Group S2) of garlic macerated oil to the lesion experimentally created lesion on the cecum serosa (Table 1). After the postoperative processes, the subjects were followed in individual cages.

**Table 1.** Study Groups and group numbers

| STUDY GROUPS | | **Total** (n) |
|---|---|---|
| **Control Group** The physiological saline solution control group *(0.9% isotonic sodium chloride i.v. infusion solution, Polifarma® Medical, Tekirdağ-Türkiye)* | **Group K** (1 ml) | 4 |
| **Experimental Groups** Macerated oil prepared with olive oil *(Zeo® Extra Virgin Olive Oil, İstanbul-Türkiye)* and 12 g fresh Taşköprü Garlic | **Group S1** (0.5 ml) | 2 |
| | **Group S2** (1 ml) | 2 |
| | | 8 |

## 2.3. Preparation of Macerated Lipogenic Garlic Extract

Before the in vivo experiment phase, the macerated garlic oil was prepared. For this purpose, Taşköprü garlic with medium sized cloves that were acquired from a local farmer with a geographical designation license were used. Commercial olive oil (Zeo® Extra Virgin Olive Oil, İstanbul-Turkey) purchased from the market and determined to be suitable by making use of chromatographic and free fatty acid analyses were used as base oil. Using 0.1 N KOH titration, the free fatty acid concentration of olive oil was found to be 0.8% (oleic acid). Since the amount of free fatty acid was found to be lower than 1% accepted to be the lower threshold for saponification, no additional process was performed before the use [15]. Macerated oils were obtained using garlic having different weights and, considering the potential side effects of garlic, macerated garlic was prepared with olive oil by using 12 g garlic that was the minimum weight providing the most suitable results in Fourier transform infrared spectrophotometer (FTIR) analysis. For this purpose, garlic cloves were peeled and dissected into small pieces. Prepared macerated oil using fresh garlic at a concentration of 1.2 g/ml in olive oil was put into an amber-colored bottle to eliminate the effect of light [12, 14]. The mixture of garlic and olive oil was incubated under darkness and at room temperature for 30 days. At the end of this period, the mixture was filtered using qualitative filter paper and garlic particles were removed. The filtrate was labeled as Z12 and the chemical analyses were performed. Until the in vivo use, the resultant macerated oil was kept at 4°C by sterilizing using a sterile injector filter (0.22 µm, CA). The experiments were conducted in Biomedical Engineering Tissue Culture Laboratory and analyses were performed in Kastamonu University's Central Research Laboratory Center of Application and Research.

## 2.4. Chemical Analysis of Oil Using FTIR

The structure of chemical components in the Z12 oil sample was analyzed using Bruker® brand Alpha Model ATR-FTIR spectroscopy device (USA). Measurements were performed between 4000 and 400 $cm^{-1}$ at 4 $cm^{-1}$ resolution with 24 scanning parameters.

## 2.5. Characterization of Oil Composition

The chemical components of macerated oil and their amounts were analyzed using chromatographic methods. First, the essential oil analysis was conducted using gas chromatography-mass spectroscopy (GC-MS) (Shimadzu GCMS-QP2010 ULTRA, Japan). The analysis was conducted for 63 minutes with 3 minutes at 40˚C temperature, 4˚C/min. increase from 40˚C to 240˚C, and 10 minutes at 240˚C. The results were scanned using the device library and components were matched.

Full component analysis of Z12 sample was performed using liquid chromatography-mass spectroscopy/mass spectroscopy (LC MS/MS) (Shimadzu LCMS-8040, Japan) device that can perform more accurate analysis and detect also non-essential oils. During the analysis, measurements were performed using the parameters of the Q3 Scan method, column-free injection, analysis time of 1 minute, injection volume of 0.2 µl, ultrapure water containing 1‰ formic acid (Mobile phase A), and methanol containing 1% formic acid (Mobile phase B). Peaks detected in the chromatogram of the Z12 sample were determined by reviewing and using the literature and PubMed database.

## 2.6. Creation of Cecal Abrasion

The subjects in all the groups were taken under general anesthesia by the intra-peritoneal implementation of a 2.5% concentration of Tribromoetanol at the dose of 250 mg/kg [20]. The subjects were positioned in a dorsal position. The hairs on the surface of the abdomen were shaved and, after asepsis-antisepsis, laparotomy was performed with an approximately 2 cm median line incision under sterile conditions. After removing the cecum (Figure 1A), cecal abrasion was applied on the upper surface by using a sterile gauze (Figure 1B-1C) [9]. For the subjects in the control group, 1 ml sterile physiological saline solution was dropped on the abrasion zone and the laparotomy incision was closed. Among subjects in the experimental group, two were given 0.5 ml (S1) and the other two were given 1 ml (S2) of sterile macerated oil (Figure 1D) and then the incision line was closed with surgical sutures (Surgicryl® polyglactin 3/0, SMI Steinerberg-Belgium) routinely. To prevent any postoperative complications due to liquid (blood) loss during the postoperative process, all the subjects were received isotonic sodium chloride (0.9%) subcutaneously at the dose of 5 ml/kg as prophylactic liquid support [21]. All the surgical interventions were performed by the same researchers**.**

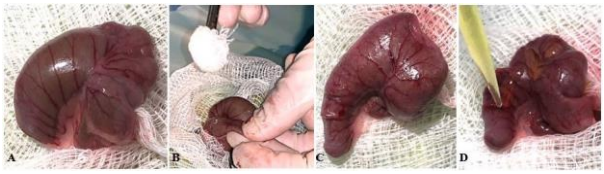


**Figure 1. A.** Cecum was taken out of the abdomen, **B.** Application of abrasion on the upper surface of the cecum by using sterile gauze, **C.** The appearance of cecum serosa after abrasion application, **D.** Application of sterile macerated oil (Z12) to cecum serosa

**Table 2.** Macroscopic and histopathological assessment criteria for intra-abdominal adhesions

| Grade/ Score | Macroscopic Assessment Criteria* | Histopathological Assessment Criteria** |
|---|---|---|
| 0 | No adhesion | No fibrosis |
| 1 | Very thin and easily separable adhesions | Thin cellular fibrous bundles |
| 2 | Thick adhesions limited to a specific region | Wide fibrous areas with decreased vascularization |
| 3 | Thick and widespread adhesions | Fibrous areas constituted by thick collagen bundles |
| 4 | Thick and widespread adhesions and adhesions observed between abdominal organs and the abdominal wall | - |

*Blauer and Collins, 1988, **Yılmaz et al., 2005

### 2.7. Clinical Assessment

In the postoperative period after the experimental intervention, all the subjects were examined clinically four times a day for the first 3 days and 2 times for the remainder in terms of water consumption, defecation, respiration, mobility, and wound status, and the findings were recorded on the follow-up notebook.

### 2.8. Macroscopic and Histopathological Adhesion Scoring

Two animals from each group were put down on the 3rd and 28th postoperative days and the adhesions determined during the intra-abdominal exploration were macroscopically assessed/scored using the criteria of Blauer & Collins (1988) (Table 2).

The samples obtained from the subjects were found to have adhesion, staining was performed using hematoxylin-eosin (H&E) for cells, safranine-O (SO) for all the protein content including collagen, and alcian blue (AC) for proteoglycans [22]. Given the staining results, the adhesions were rated between 0 and 3 according to the criteria of Yılmaz et al. (2005) (Table 2).

## 3. RESULTS and DISCUSSION

### 3.1. FTIR Analysis

FTIR spectra of the Z12 sample indicated the presence of functional groups such as hydroxyl, carbonyl, carboxylic, and organosulfur compounds. However, the extracts prepared exhibited peaks mainly specific to the olive oil. Besides that, the weak C=S elastic vibrations at 1200-1050 $cm^{-1}$ suggest the presence of C-N stretching of primary amines and organosulfur compounds including allyne, allicin, and diallyl disulfide (Figure 2). The weak peak observed at approximately 1350 $cm^{-1}$ indicates the -SO2- (sulfone) groups. Moreover, the differences in fingerprint zone arise from the garlic extracts put into the olive oil. In this region, the peaks at 630-650 $cm^{-1}$ indicate the C-S bound flexion [24].



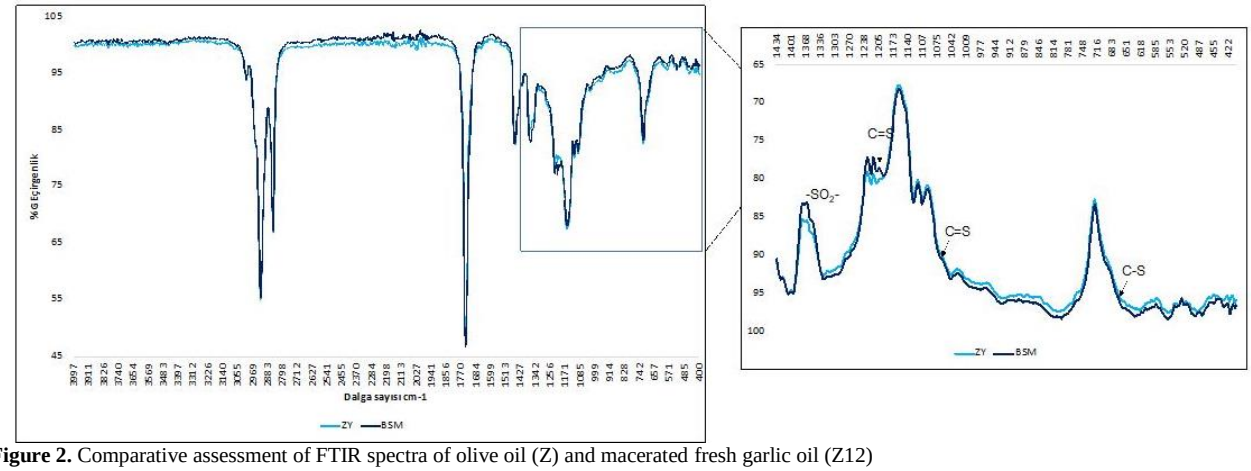


**Figure 2.** Comparative assessment of FTIR spectra of olive oil (Z) and macerated fresh garlic oil (Z12)

### 3.2. Component Analysis with LC-MS/MS

Mainly the olive oil components were observed in LC-MS/MS analysis performed for the Z12 sample. In the positive ion scanning, no component that was specific to garlic could be found. However, the chromatogram illustrating the garlic-specific components detected in negative ion scanning [25, 26] is presented in Figure 3.

Through the enzymatic reaction induced by the crush of garlic cells and their exposure to air, the alliinase enzyme interacts with alliin and forms allicin [10, 11,13, 14]. The chemical composition of macerated garlic is related to the possible substrate activity of allinase enzyme within the garlic cell, the use of polar and/or non-polar extraction solvents, and the maceration conditions [14]. Hence, in their study, Ferioli et al. (2020) detected a high amount of lipophilic organosulfur compounds in sunflower oil extracts having an apolar character. In the same study, the analyses conducted with commercially available products suggested that commercially available oil extracts might not contain almost any bioactive compound at all. The presence of various products specified for different purposes makes the standardization of garlic products difficult. Because of the uncertainties in products, it was clearly emphasized that it is important to reveal the sulfurous compounds and their amounts in a commercially available garlic product before the use for potential beneficial effects on health [14]. From this aspect, it is thought that the difference between the results reported in previous studies examining the adhesion-prevention effect of macerated garlic oil [3, 4, 7] arose

from the use of commercial macerated oils manufactured by different companies and have different chemical compositions. This thought is also corroborated by the fact that oils used in those studies were not analyzed for suitability. The macerated garlic oil used in the present study was prepared by us by using commercially available olive oil, the suitability of which was confirmed, and locally produced original Taşköprü Garlic.

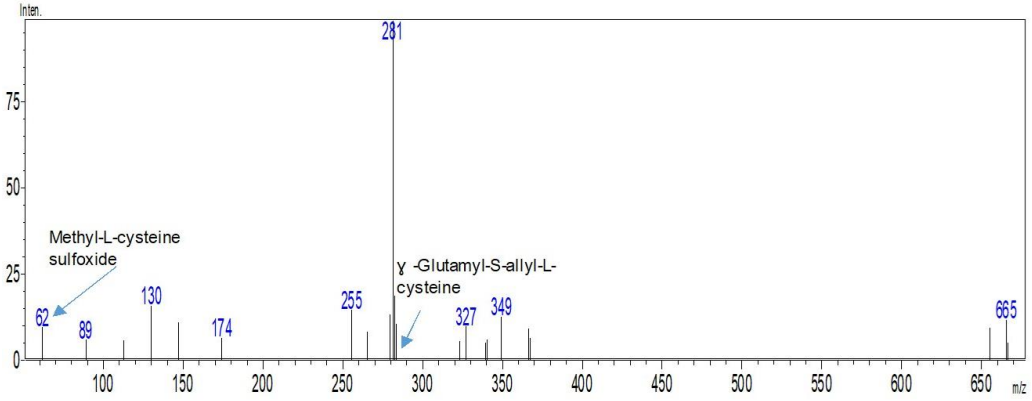


**Figure 3.** Chromatogram of Z12 sample's LC-MS/MS negative ion

During the preparation phase, various composition ratios were tested (only the data of extract that was used were presented due to a large amount of data) and the macerated oil containing the minimum amount of extract was preferred considering the potential adverse effects of garlic. The chemical structure of the macerated oil, prepared using fresh garlic at a concentration of 1.2 g/ml in olive oil, was analyzed spectrophotometrically using FTIR first. FTIR spectroscopy clearly showed the vibrations, which arise from the shifting movements of links between the atoms constituting functional groups, in mid-infrared range (MIR). The peaks belonging to sulfurous structures that are active compounds specific to garlic were at low levels between 1300 and 1050 $cm^{-1}$ and there was a C-S link flexion in the fingerprint region. These findings confirm the transition of garlic extracts into olive oil. Chromatographic analyses were performed to determine the name and chemical formula of garlic components in the Z12 extract, the suitability of which was determined using FTIR. In the analysis performed using GC-MS, no essential oils were found but the components that were specific to olive oil were detected. Among the components detected during the more precise measurements performed using FTIR, the essential oils were below the detection limits of GC-MS. Thus, LC-MS/MS analysis was performed in order to determine all the components of the macerated mixture. Garlic specific carbohydrates and sulfurous components were detected during the LC-MS/MS analysis and it was determined that macerated oil had bioactive characteristics that were specific to fresh garlic. After these results, the in vivo use of macerated garlic oil was initiated.

### 3.3. Essential Oil Analysis with GC-MS

The essential oils of Z12 specific to garlic and observed at a low level in the FTIR spectrum couldn't be detected with GC-MS. The components detected were generally those of olive oil (Table 3) and it is thought that garlic extracts were not observed in the chromatogram since they were in trace amounts.

**Table 3.** Chemical composition of macerated garlic (*Allium sativum L.*) oil as a result of GC-MS analysis

| No | Retention time | Compounds | % | ID |
|---|---|---|---|---|
| 1 | 10.612 | Octanoic acid, methyl ester (CAS) | 0.845 | GC/MS |
| 2 | 24.744 | Tetradecanoic acid, methyl ester (CAS) | 1.718 | GC/MS |
| 3 | 28.977 | Palmitate <methyl-> | 310.679 | GC/MS |
| 4 | 30.099 | 9-Hexadecenoic acid, methyl ester, (Z)- | 17.324 | GC/MS |
| 5 | 30.912 | Heptadecanoic acid, methyl ester (CAS) | 3.300 | GC/MS |
| 6 | 32.801 | Methyl stearate | 49.978 | GC/MS |
| 7 | 33.760 | 9-Octadecenoic acid (Z)-, methyl ester (CAS) | 2108.934 | GC/MS |
| 8 | 35.147 | 9,12-Octadecadienoic acid (Z, Z)-, methyl ester | 212.660 | GC/MS |
| 9 | 36.232 | Eicosanoic acid, methyl ester (CAS) | 7.528 | GC/MS |
| 10 | 36.793 | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z) | 9.749 | GC/MS |
| 11 | 37.099 | cis-11-Eicosenoic acid, methyl ester | 5.606 | GC/MS |
| 12 | 37.867 | Heneicosanoic acid, methyl ester (CAS) | 1.753 | GC/MS |
| 13 | 39.430 | Docosanoic acid, methyl ester | 3.042 | GC/MS |
| 14 | 42.569 | Tetracosanoic acid, methyl ester | 2.135 | GC/MS |

### 3.4. Clinical Assessment

During the postoperative clinical observations in terms of feed-water consumption, defecation, respiration, mobility, and wound condition after waking up from anesthesia, it was determined that the subjects that turned back to daily activities within six hours approximately, and they were generally in good condition.

### 3.5. Adhesion Evaluation

#### 3.5.1. Macroscopic adhesion scoring

The distribution of adhesions rated between 0 and 4 according to macroscopic adhesion score (MAS) using

the criteria of Blauer & Collins (1988) on postoperative days 3-28 is presented in Table 4, whereas the images related to the MAS assessments are illustrated in Figure 4.

**Table 4.** MAS and HAS assessments on the 3rd and 28th postoperative days

| GROUPS | Day 3 | | Day 28 | | *Total (n)* |
|---|---|---|---|---|---|
| | MAS | HAS | MAS | HAS | |
| K * | 1<br>2 | 1<br>1 | 2<br>0 | 2<br>0 | 4 |
| S1** | 1 | 1 | 0 | 0 | 2 |
| S2*** | 1 | 1 | 0 | 0 | 2 |
| *Total (n)* | 4 | | 4 | | 8 |

* Physiological saline solution group
** Group treated with 0.5 ml dose of macerated oil
*** Group treated with 1 ml dose of macerated oil

Given the macroscopic assessments performed in the present study, all the subjects were found to have an adhesion on the 3rd day. Moreover, it was observed that adhesions reached the level of MAS-2 in the control group but only MAS-1 level adhesions were observed in the experimental group subjects. On the 28th day, no adhesion was observed in the experimental group subjects but the level of adhesions seen in the control group subjects was MAS-2. In all the time points and all experimental group subjects (S1-S2), the abdominal exploration showed that the color of cecum serosa was bright and smooth and turned into dark green (Figure 4). It was thought that the change in the color of cecum serosa in experimental group subjects during the postoperative period was related to the topical sensitivity due to the garlic [14].

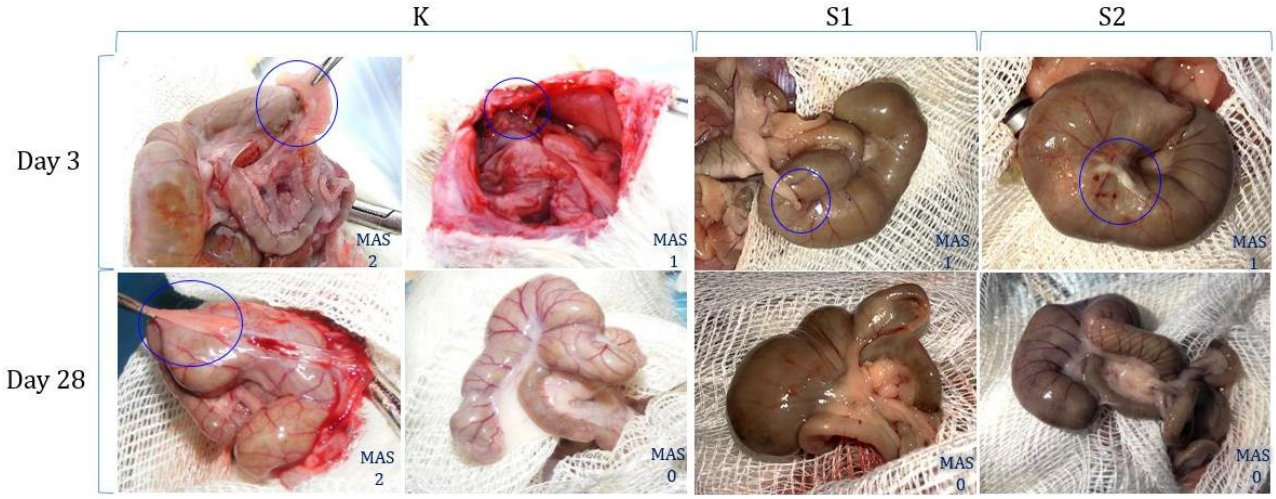


**Figure 4.** Images and MAS values of control (K) and experimental groups [S1 (0.5 ml)-S2 (1 ml)] on postoperative days 3-28

It was emphasized that the synergetic or antagonistic effect of garlic products on physiology might vary depending on dosage, potential metabolite interactions, and the individual's age and pathology. In humans, excessive consumption of garlic might cause various adverse effects such as allergic reactions, topical sensitivities, prolonged blood coagulation [14], unpleasant odor in breath and on the skin, stomach problems such as indigestion, hypotension [10, 13, 14], tachycardia, headache, sleeplessness, vomiting, and diarrhea. Since it increases the effects of anticoagulant agents (warfarin, coumadin, fluindione, etc.), it is not recommended to use garlic together with these medications [10, 13]. Moreover, it was also reported that consuming a high amount of garlic after surgical interventions increased the risk of postoperative hemorrhage [10]. In studies reporting that intra-peritoneal use of garlic oil reduced the formation of peritoneal adhesion [3, 7], garlic oil was used at the dose of 5 ml/kg. But it was also stated that more experimental studies are needed to determine the best dose of garlic offering the best adhesion prevention [7]. It was aimed to determine the active component content of macerated oil and if it would be effective at that dose because no commercially available preparation was used and because of the presence of bioactive components of garlic detected in laboratory analyses. From this aspect, the macerated oil containing the minimum concentration (1.2 g/ml) of garlic was applied to the cecum serosa of the subjects; the dose was 1 ml (5 ml/kg) for half (n=2) of subjects and 0.5 ml (2.5 ml/kg) for the other half (n=2).

### 3.5.2. Histopathological adhesion scoring and assessment

The histopathological adhesion scores (HAS) of adhesions rated histopathologically between 0 and 3 using the criteria of Yılmaz et al. (2005) are presented in Table 4.

The distribution of adhesions rated histopathologically between 0 and 3 [23] by HAS is presented in Figure 5 together with the histochemical images. In the control group given physiological saline solution, adhesion formation due to cellular fibrosis was observed on day 3. Given the SO staining in the matrix within adhesion tissue, there were fewer protein structures, whereas AC stating showed that there were more proteoglycan molecules. Especially the glycosaminoglycan (GAG) structures were in form of small aggregates gathering nearby the cells and they were observed to be more in accordance with the cell density. The results obtained from the control group on the 28th day revealed that cellular fibrosis started decreasing but, together with the

increased protein level, SO staining indicated the formation of fibrillary collagen bundles. In this parallel, H&E and AC staining procedures showed relative decreases. Moreover, AC staining procedures revealed that, rather than aggregating nearby the cells, proteoglycan-type structures exhibited a fibrillary alignment throughout the matrix. Considering all three histochemical staining processes together, HAS in the control group was rated 1 on day 3 and 2 on day 28.

In the experimental groups (S1-S2), there was cellular fibrosis but at a lower level in comparison to the control group. H&E and AC results corroborated each other and GAG aggregates gathered around the cells. In the S2 sample, proteoglycans were found to shift toward the intercellular area. SO staining procedure stains proteins in the extracellular matrix. SO results in S1 and S2 showed that SO stained the core proteins belonging to proteoglycans concentrated around the cells rather than fibrous proteins (collagen molecules). Considering all three histochemical staining procedures, S1 and S2 samples were found to be HAS-1 on day 3 [23]. Since no adhesion tissue formed on day 28 in the S1 and S2 samples, HAS assessment been considered to be zero.

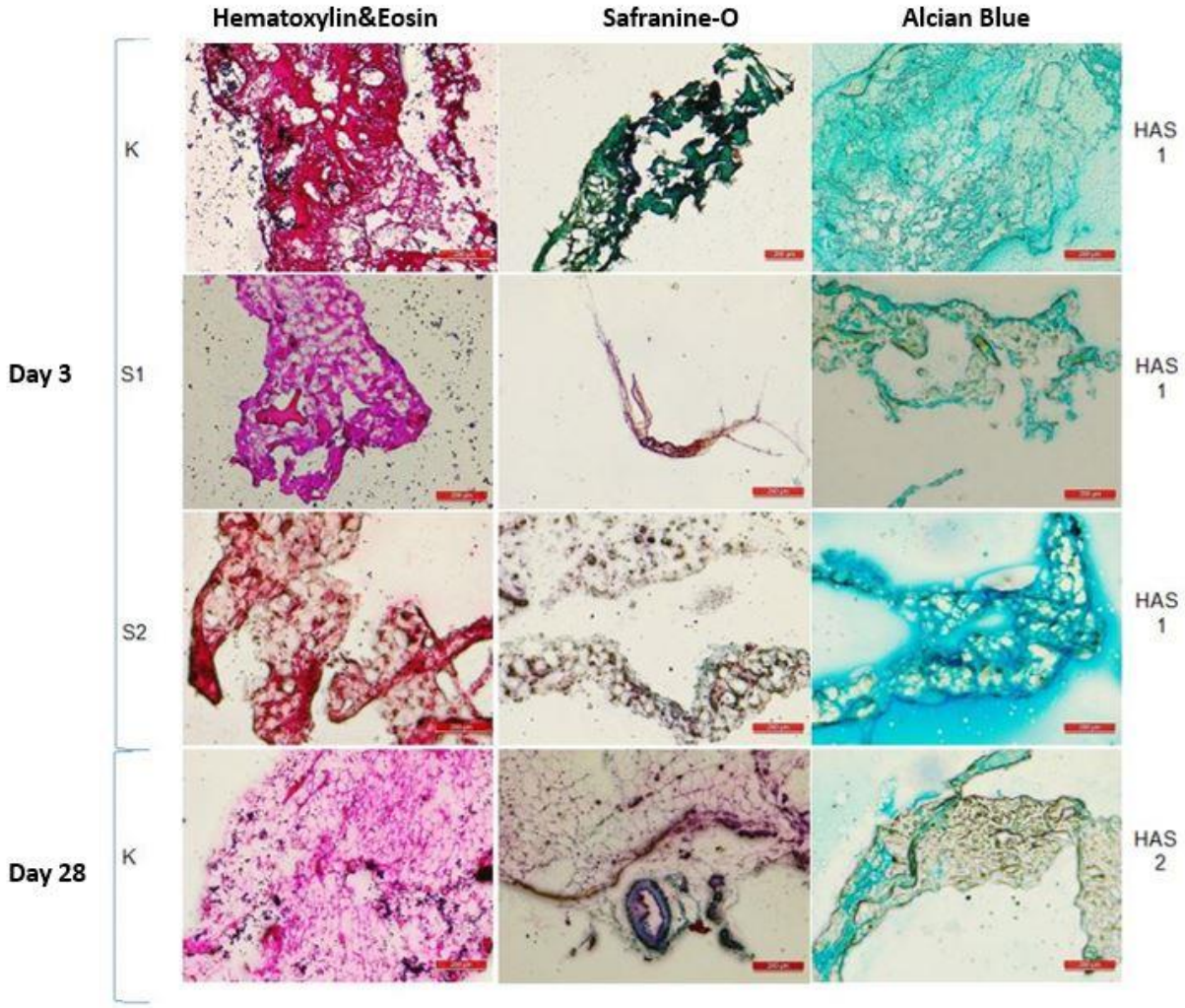


**Figure 5.** HAS assessment and histopathological examinations were performed using H&E, SO, and AC staining. Since no adhesion tissue formed in S1 and S2 samples on day 28, HAS assessment was considered to be zero (scale = 200 µm)

Ali et al. (2016) reported that intra-peritoneal implementation of garlic oil in diabetic and non-diabetic rats was effective in reducing postoperative adhesions, especially in diabetic subjects. This effect was related to the antimicrobial and anti-inflammatory activity of garlic and the prevention of mechanical contact between surfaces [7]. In a study by Topal et al. (2019), the immunohistochemical analyses conducted to determine the oxidative stress on the cellular surface revealed that garlic didn't reduce the free oxygen radicals. Besides that, in another study [7] analyzing the intra-peritoneal use of garlic oil, immunohistochemical examinations showed that there was no significant increase in D2-40 positive cells in control groups and those given physiological saline solution, whereas positive immune reactions to D2-40 antibodies were found in mesothelium cells in the groups treated with garlic oil. The diabetic rats treated with garlic oil had significantly increased D2-40 expression when compared to those treated with physiological saline solution and the difference was found to be statistically significant ($p<0.001$) [7].

Macerated oil of fresh garlic, which was found to increase the proliferation in human cancer cells and rat bone marrow mesenchymal stem cells (MSCs), was thought to exhibit a nutritive effect on the cells because of the oleic

acid content of pure olive oil [7]. During the in vitro cell culture studies carried out in our laboratory, it was determined that pure olive oil increased the proliferation in all the healthy cells and/or those having pathological phenotypes and, thus, promoted tissue formation [15]. The oleic acid content in the pure olive oil used as the base oil in the present study might support the proliferation of fibroblast cells in adhesion structure as in MSCs having a fibroblastic morphology. However, it was observed that the adhesion ratio in the macerated fresh garlic oil group was lower in comparison to the control group. This finding was interpreted as the antiproliferative effect of garlic extracts. Given the MAS values determined on the 3rd and 28th postoperative days, experimental groups (S1-S2) were found to have macroscopically less and lower levels of adhesions in comparison to the control group. Besides that, the histopathological lesion that was at HAS-1 level, which was related to cellular fibrosis, in all the groups on day 3 was found to be at HAS-2 level, which was related to the fibrous tissue-like structures increasing against the decreasing cellular structure, in the control group on day 28. However, no adhesion tissue was observed in S1-S2 subjects on day 28. This result suggested that either no adhesion has formed or the low levels of adhesions observed on day 3 were eliminated by the body with time.

In the present study, the small number of subjects examined at the time points is a limiting factor. For this reason, further studies using garlic, the bioactive components of which were determined, and to be carried out on a higher number of subjects are needed to determine the minimum dose of garlic oil to be applied intra-peritoneally to prevent the adhesion.

## 4. CONCLUSION

It was thought that the macerated oil of fresh garlic, which was prepared using olive oil having low oleic acid and free fatty acid as base oil and bioactive components which were determined as in the present study, would be more effective in preventing adhesion. Besides that, garlic having a high level of sulfur content, which is known to play role in antioxidant and antimicrobial activity, had a significant contribution to the result obtained. In the present study, it was concluded that the intra-abdominal application of macerated olive oil obtained from fresh fresh garlic was effective in preventing postoperative adhesion. It may be easily performed in veterinary clinic practice. To prevent the formation of intra-abdominal adhesions and to determine the effect mechanism of macerated oil that was applied, there should be further postoperative studies should be carried out on a higher number of subjects and in shorter time points.

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