

RESEARCH ARTICLE

Protective Efficacy of Fresh and Aged Macerated Garlic Oils in Safflower Oil Against Intra-Abdominal Adhesions in Rats

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ABSTRACT

This study aims to investigate the protective efficacy of fresh (FG) and aged (AG) Taşköprü Garlic (*Allium sativum L.*) macerated oils prepared in safflower oil at different concentrations against post-operative (PO) intra-abdominal adhesions (PIA). No commercial garlic product was used in the study. AG was produced from Taşköprü Garlic, which is one of the geographically indicated products of the European Union. Then FG and AG were kept at two different concentrations (1.5 g/mL & 2.0 g/mL) in safflower (*Carthamus tinctorius L.*) oil to get macerated oils of FG15, FG20, AG15 and AG20. After spectrophotometric and chromatographic analyzes of the oils, *in vivo* experiments were made on rats. A cecal abrasion model was applied to six study groups viz FG15, FG20, AG15, AG20, serum physiologic control and oil control. Their preventive activities against PIA were examined macroscopically and histopathologically at different time points (7th-14th-21st days PO). The safflower oil did not give successful results in preventing PIA when used alone. The AG macerated oils, especially AG20, caused negative effects on the injured tissue/organ surfaces due to their high ratio of 2,4-decadienal content. The macerated oils prepared with fresh Taşköprü Garlic in safflower oil (FG15 and FG20) at the rate of 0.1 mL were found successful agents to prevent PIA by intra-abdominal route without any irritant effect in rats. These oils are considered as low cost clinical/laboratory preparations and can be used in laparotomy/laparoscopy to prevent PIA.

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INTRODUCTION

Post-operative intra-abdominal adhesions (PIA) are very important complications after surgical procedures (Brochhausen *et al.*, 2011; Köm, 2013). Lots of strategies/agents have been investigated against PIA such as surgical techniques, mechanical barriers and chemicals but pre-clinical/clinical studies are still necessary (Brochhausen *et al.*, 2011; Aysan *et al.*, 2012).

Garlic has been used traditionally to treat so many diseases/conditions such as infections, wounds, cardiovascular diseases since ancient times (Ferioli *et al.*, 2020) and an interest in alternative garlic products has been increased (Lawson, 1998). Garlic oil has a wide range of pharmacological properties, such as anticarcinogenic, antibacterial, anti-inflammatory, fibrinolytic, wound healing, antioxidant and antiadhesive activities. It is known as an effective agent as protective and anti-inflammatory on tissue damage caused by corrosive burns (Tanrikulu *et*

al., 2017). Allicin is accepted the basic active component in the garlic (Lawson, 1998; Zor, 2006). Macerated garlic oil contains unique sulfur compounds such as ajoene, vinylthiols and allyl sulfides, which are formed by the conversion of allicin (Staba *et al.*, 2001; Yoo *et al.*, 2014; Patil and Ravindra, 2008; Ferioli *et al.*, 2020). Ajoenes reducing platelet aggregation (Jiang, 2019) can remain undisturbed in garlic macerated oil for a long time (Staba *et al.*, 2001; Patil and Ravindra, 2008). Similar to garlic, safflower oil also has cardio-protective, antioxidant (Smith, 2005; Khalid *et al.*, 2017) and *in vitro* platelet aggregation properties (Smith, 2005).

Developing the effective adhesion prevention strategies could reduce adhesions morbidity and management costs (Arung *et al.*, 2011). The application of high viscosity oils to prevent PIA has been stated as a simple and cost-effective approach (Aysan *et al.*, 2012). The garlic and the safflower oil have bioactive properties on fibrinolytic system due to antiplatelet aggregation activity.

Therefore, it was aimed to investigate the preventive effects against PIA by the FG and AG macerated oils prepared at different concentrations in safflower oil.

MATERIALS AND METHODS

Ethical statement: This study was performed with the approval of the Animal Experiments Local Ethical Committee of the Kastamonu University via reference No. 2019/23(2021-10) and 2020/08(2021-08).

Preparation of macerated oils: Aged garlic were produced from FG by a hydro-thermal process in the laboratory. Then, crushed FG and AG were put separately into the safflower oil and kept at room temperature in the dark for 20 days. Garlic macerated oils were prepared at two different concentrations i.e. 1.5 g/mL and 2.0 g/mL (Patil and Ravindra, 2008). The macerated oils were filtered by a sterile injector filter (pore size 0.22 μm) and stored in the dark at 4°C (Yoo *et al.*, 2014).

Analysis of garlic macerated oil: The analyses of prepared macerated oils were carried out in the Kastamonu University Central Research Laboratory through standard protocols. Briefly, the macerated samples were analyzed with Fourier Transform Infrared Spectrophotometer (FTIR) with Bruker® Brand alpha model ATR-FTIR spectroscopy device (Billerica/USA) used at 4000-400 cm^{-1} (Nagarajan and Kumar, 2017). Samples were also analyzed with the gas chromatography-mass spectroscopy (GC-MS) using by Shimadzu® GC-MS QP 2010 ULTRA instrument (Shimadzu Corporation/Japan) (Alshawi, 2021). The results were compared with the device library.

Experimental animals: A total of 18 Wistar Albino rats (weighing 200-300 g and 3-6 months old) were included in the study. Rats were housed in individual cages at room temperature (19-22°C), 12 hours darkness/12 hours daylight and 45-55% humidity level. They were fed ad libitum with standard pelleted food and water. Experimental rates were divided into six groups: four treated groups (FG15, FG20, AG15, AG20) and two control (C1, C2) with 3 rats in each group.

Creation of an abrasion lesion on cecum: An abrasion lesion on the cecum of all experimental groups was created through laparotomy. After the incision, the anti-mesenteric side of the cecal serosa was rubbed until slight bleeding appeared. Different substances (macerated oils, safflower oil and saline) were dripped on the abraded surface in each group at 0.1 mL dosage. Then the cecum was replaced, laparotomy incision line was closed with the routine surgical sutures (Surgycryl® PGA, 3/0, SMİ, Belgium). All surgical procedures were performed under general anesthesia (Tribromoethanol, 1.25-2.5%, 300 mg/kg, intraperitoneal) as per guidelines of Washington State University IACUC, 2019.

Postoperative care: All the experimental rats were inspected for respiration, movement, feed/water consumption and defecation four times a day in the first 3 days, then twice a day in the remaining days. The status of the wounds was also checked on each visit.

Macroscopic and histopathological adhesion scoring:

Re-laparotomies were performed on 7th-14th-21st days PO for macroscopic adhesion scoring (MAS) and histopathological adhesion scoring (HAS) as depicted in Table 1. Under general anesthesia, the tissue samples were taken for histopathological examinations from the adhesion sites. The frozen section method (Leica® CM 1860 UV cryo-microtome-Germany) was used for histopathology (Dey, 2018). The sample sections were stained according to standard protocols and examined under a light microscope (Leica® DMIL 100, Germany). The collagen fibers were detected by staining with Safranin-O, cells with hematoxylin-eosin (H&E), and proteoglycans with alcian blue stains (Yazar, 2022).

Statistical analyses: The grading of adhesions was compared by the Chi-square test and according to results Fisher's Exact Test or Pearson Chi Square was taken into account. P value less than 0.05 was considered significant. All statistical analyses were conducted by SPSS statistical program (version 23).

RESULTS

FTIR analysis: In the FTIR spectra of the garlic macerated oil samples, safflower oil-specific peaks were observed generally (Fig. 1). A partial increase was observed during FG, AG around 1372 cm^{-1} in the intensity of weak peaks showing C=S stretching in mainly sulfur compounds. Similarly, a partial increase was observed during FG and AG in the peaks of C-N stretching in mainly amino acids at 1233 cm^{-1} . The peak at 1058-982 cm^{-1} in the AG and 1029-976 cm^{-1} in the FG samples reveals the presence of C-N stretches of primary amines, symmetric C-H stretching vibration presence of antioxidant enzymes and organosulfur compounds including alliin, allicin and diallyl-disulfide containing the S=O group. The peak, seen at 876 cm^{-1} in both samples, can be attributed to the C-H deformation of =CH₂, while the peak that was seen at 913 cm^{-1} indicates the N-H vibration of the primary amine curves of the amine group of alliin.

GC-MS analysis results: The garlic-specific sulfur compounds were found in certain proportions in FG macerated oils (Table 2). However, garlic-specific components could not be determined in the AG macerated oils. The garlic-specific trisulfide components were found 1.51 and 1.98% in FG15 and FG20, respectively. These ratios showed that the total trisulfide compounds in the garlic were extracted in the base oil in the amount of 2.75 and 3.61%. The ratio of total diallyl-sulfides was similar in FG15 and FG20 and measured as 17.43 and 16.83%, respectively. It was evident that diallyl-sulfides leached in the base oil of FG15 and FG20 at a rate of 39.13 and 37.79%, respectively. Besides, 2,4-decadienal has been detected in certain amounts in macerated oils prepared using fresh and thermally processed fermented garlic (AG) in safflower oil. Approximately, the same amount of 2,4-decadienal was detected in each AG macerated oil samples (AG15, AG20). This ratio was higher by 4.7 times in comparison with FG macerated oils (FG15, FG20).

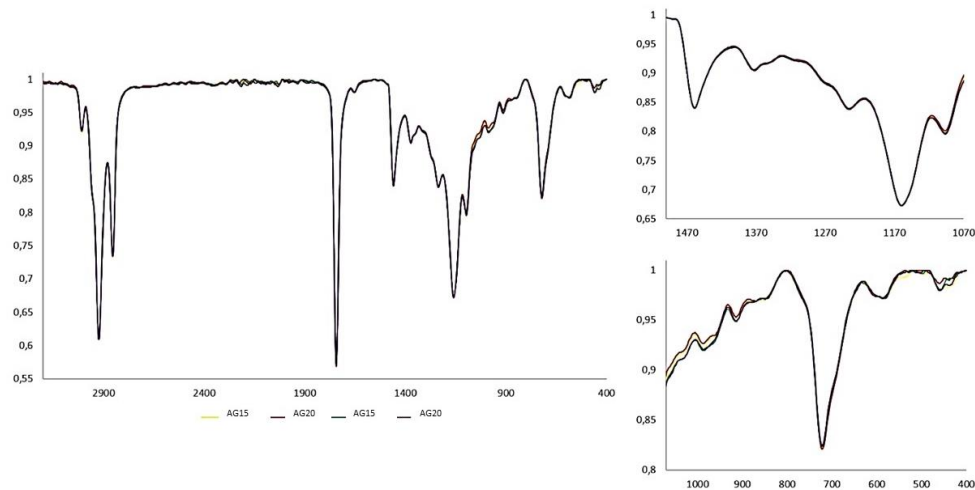


Fig. 1: Spectrums of the garlic macerated oils analyzed through Fourier Transform Infrared Spectrophotometer.

Table 1: Macroscopic and Histopathological adhesion scoring criteria of intra-abdominal adhesions according to the literatures ^{*,**}

Score	Macroscopic [*]	Score	Histopathological ^{**}
MAS-0	No adhesion	HAS-0	No fibrosis
MAS-1	Thin/narrow, easily separable adhesions	HAS-1	Thin bundles of cellular fibrosis
MAS-2	Thick adhesions limited to one area	HAS-2	Wide areas of fibrosis with reduced vascularization
MAS-3	Thick and widespread adhesions	HAS-3	Areas of fibrosis formed by thick bunch of collagen
MAS-4	Thick and widespread adhesions, adhesions of viscera to anterior and/or posterior abdominal wall	-	-

* Blauer and Collins, 1988; ** Yilmaz et al., 2005.

Table 2: The components of garlic macerated oils extracted through gas chromatography-mass spectroscopy

AREA%	NAME	AREA%
FG15	FG20	Fresh Garlic Components
2.96	3.18	Disulfide, methyl 2-propenyl
13.04	13.20	Diallyl disulphide
1.43	0.45	Tetrasulfide <diallyl->
0.47	0.71	Allyl methyl trisulfide
1.02	1.27	Allitridin
TOTAL	18.92	18.81
Safflower Oil Component		
TOTAL	6.11	6.07
	2,4-decadienal, (E,E)-	28.58
		28.94

Postoperative care: In the postoperative period, it was observed that all the rats resumed their daily activities within 6 hours and were in good general condition.

Macroscopic adhesion scoring: The intra-abdominal adhesions in the rats were scored macroscopically (Table 1) in each study group on 7th-14th-21st days PO were presented in Fig. 2 and Table 3. A very thin adhesion surface that only brought the apex and ampulla ceci closer to each other was considered as MAS-1. At all-time points, the serosa of the cecum was macroscopically bright and smooth in all treated rats except AG20.

At different time points macroscopic examinations, adhesions with different severity were detected in all groups except the FG20 group on day 7 PO. MAS-2 adhesions were seen in the C2 and AG20 groups and MAS-1 in the C1, FG15, AG15 groups. On day 14 PO, adhesion was determined as MAS-1 in the C1, C2 and AG20 groups, while there was no adhesion in all other groups. Adhesions were detected as MAS-2 level in the rats of all groups, except FG15 and FG20, on day 21 PO.

The macroscopic adhesions were determined with different levels (MAS-1 and MAS-2) in all rats (6/6, 100.0%) of the control groups (C1 and C2). The adhesion

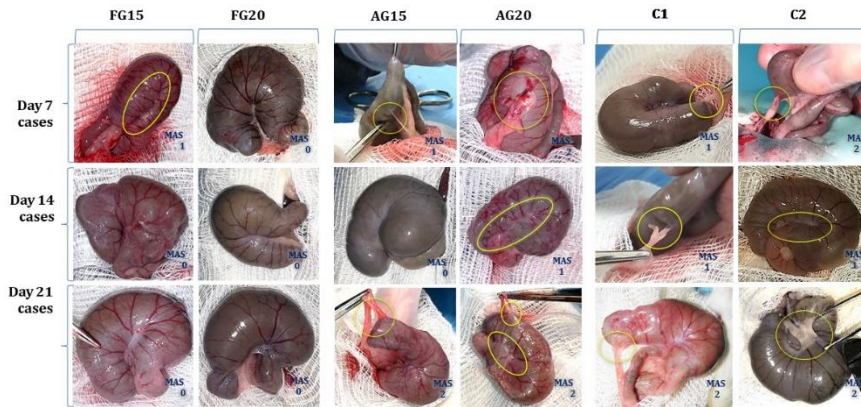
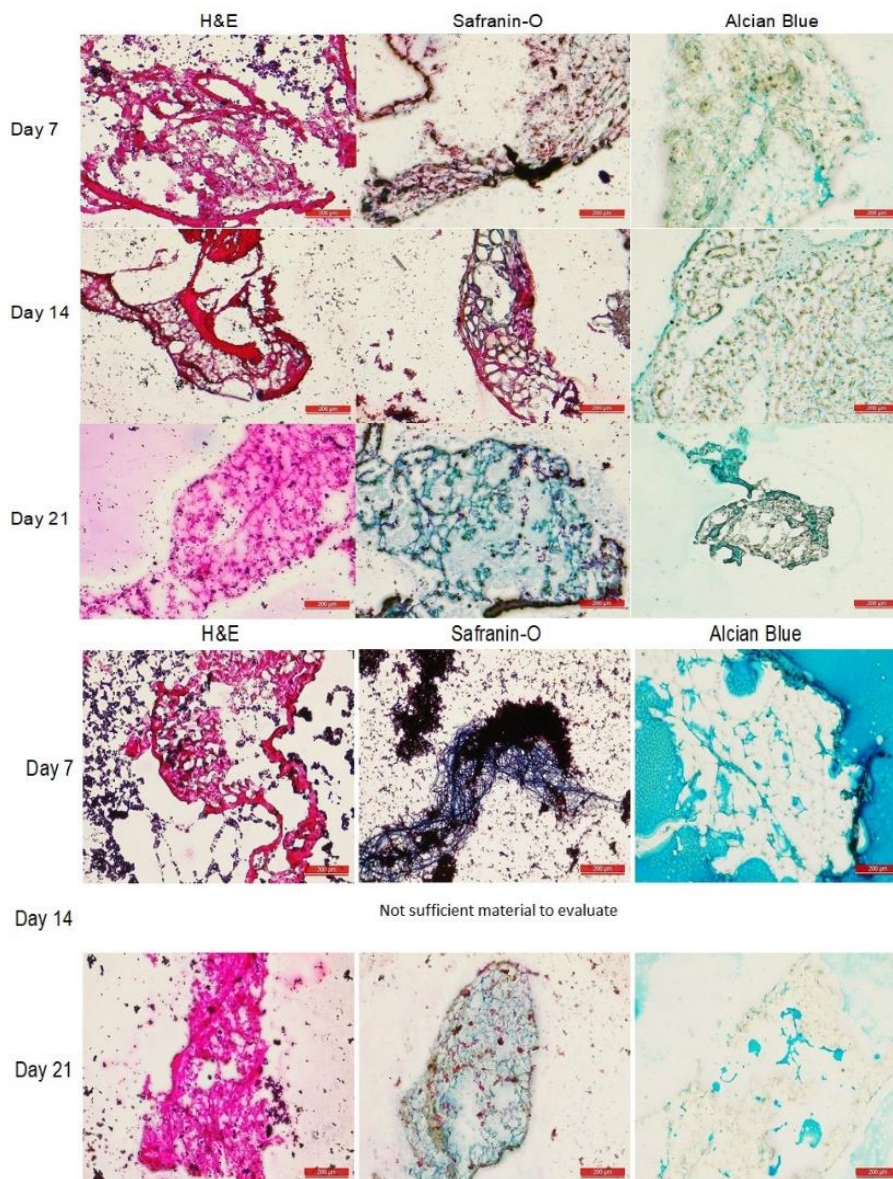
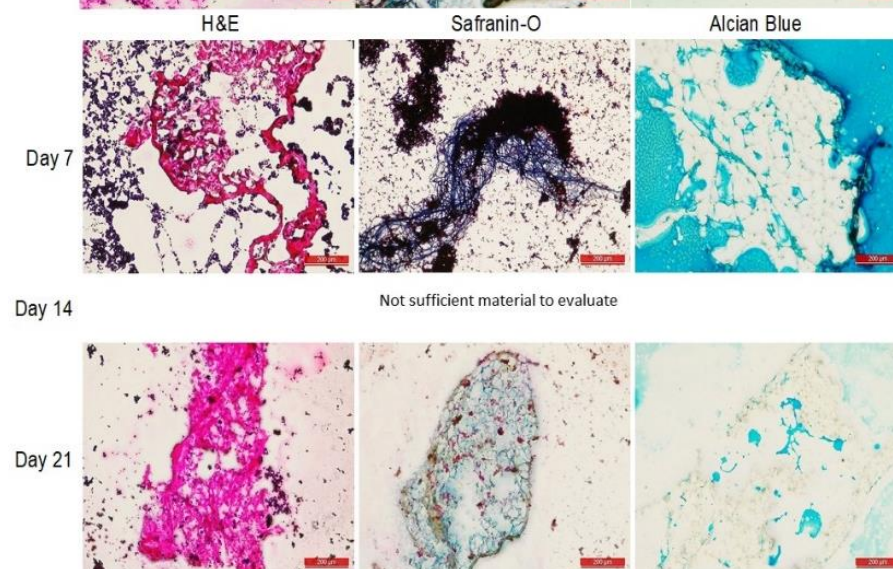
rate was determined as 50.0% (6/12) in the garlic macerated oil groups. PIA was observed in just one rat (1/6, 16.7 %) as MAS-1 degree on day 7 PO in the FG groups (FG15 and FG20). In AG groups, PIA was observed in 5 rats (5/6, 83.3%) with different levels (MAS-1 and MAS-2). It was observed as MAS-1 (1/3, 33.3%) on day 7 PO and MAS-2 (1/3, 33.3%) on day 21 PO in the AG15 group, while there was no adhesion on day 14 PO. In the AG20 group, PIA was observed in MAS-2 level on days 7 and 21 (2/3, 66.7%) and MAS-1 degree (2/3, 66.7 %) on day 14 PO. Also, the appearance of the cecal surfaces was not good at all-time points in AG20 group. On day 21, relatively thick adhesion surface between the apex and ampulla ceci with opaque differences was observed (Fig. 2). Similarly, another adhesion tissue connection was also observed on the AG20 rats where MAS was evaluated as 2.

Histopathological adhesion scoring: On day 7th-14th-21st PO, tissue samples from each experimental group were evaluated (Table 1) and presented in Table 3. On day 7 PO, the structure of the adhesion tissue was rich in cells and was evaluated as HAS-1 in group C1 (Fig. 3A). On days 14 and 21 PO, the results were similar, there was an increase in tissue thickness, so the adhesion tissue formation at both time points was evaluated as HAS-2. In addition, attachment to the intestinal wall was detected on day 14 PO, but no morphological difference was observed.

The adhesion tissue sample showed a larger matrix thickness and less cell accumulation and the experiment was scored as HAS-3 in the rats of group C2 on day 7 PO (Fig. 3B). On day 14, there was not sufficient adhesion tissue to collect a sample for histopathologic staining, hence declared as HAS-0. On day 21 PO, the tissue thickness decreased and the number of cells were higher compared to the matrix, which scored as HAS-1.

Table 3: Macroscopic and histopathological adhesion scores of various experimental groups at different time intervals (days) post-operative

		Day 7		Day 14		Day 21		Total (n)
		MAS	HAS	MAS	HAS	MAS	HAS	
CONTROL GROUPS	Group C1	1	1	1	2	2	2	3
	Group C2	2	3	1	0	2	1	3
GARLIC MACERATED OIL GROUPS	Group FG15	1	0	0	0	0	0	3
	Group FG20	0	0	0	0	0	0	3
	Group AG15	1	1	0	0	2	3	3
	Group AG20	2	0	1	0	2	0	3
Total (n)		6		6		6		18

**Fig. 2:** Macroscopic adhesion scores of treated and control groups at different time interval (days) post-operative. An opaque difference in macroscopic appearance of AG20 can be seen on day 21, postoperative.**Fig. 3A:** Histopathological analysis of saline control group (C1) samples.**Fig. 3B:** Histopathological analysis of safflower oil control group (C2) samples.

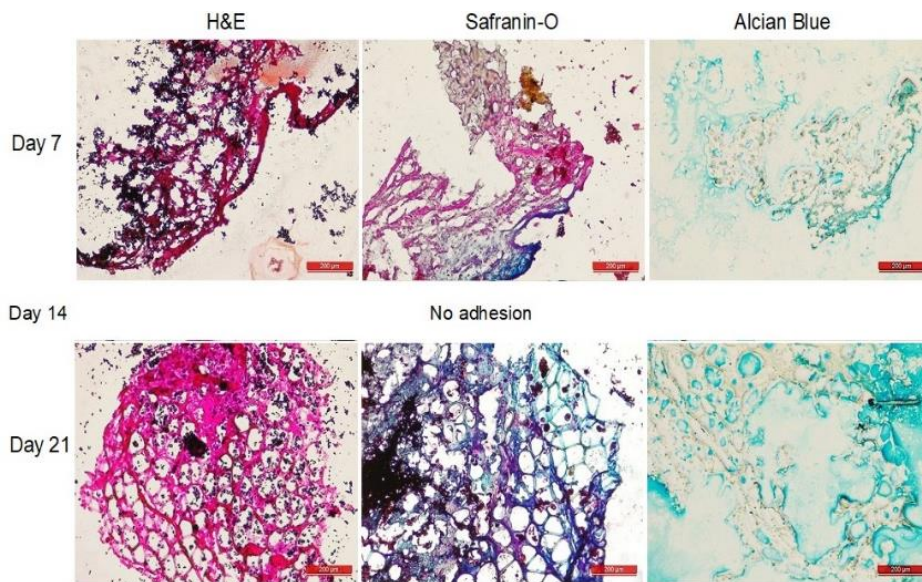


Fig. 3C: Histopathological analysis of AG15 group samples.

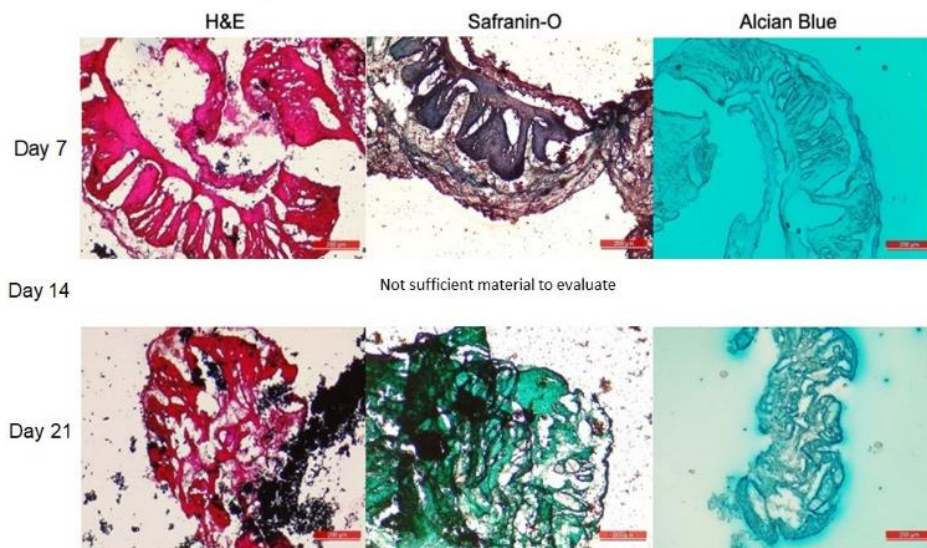


Fig. 3D: Histopathological analysis of AG20 group samples.

In group AG15, there were more cellular accumulations in the adhesion tissue on day 7 PO and that the thickness of the tissue increased due to an increase in matrix components on day 21 PO (Fig. 3C). The histopathologic adhesion severity was evaluated as HAS-1 on day 7 PO when the matrix had the least number of cells and it was evaluated as HAS-3 on day 21 PO. No adhesion was observed on day 14 PO.

Adhesions in the form of the intestines sticking together were observed in samples of the group AG20. The collected samples were the intestinal walls, not a separate adhesion tissue. Thus, the HAS of AG20 was accepted as HAS-0. On the other hand, microscopic examination revealed deterioration of the intestinal mucosa. It was observed that the morphological changes in the mucosa structure increased even more in the later days of application (Fig. 3D). Overall, no significant difference ($P>0.05$) was observed between the groups in terms of adhesion severity.

DISCUSSION

Surgical interventions are the primary causes of PIA accepted as a pathological extension of physiological

peritoneal healing (Brochhausen *et al.*, 2011; Köm, 2013). PIA may cause different complications such as chronic abdominal pain, intestinal obstruction, infertility (Köm, 2013; Şahbaz *et al.*, 2014). Although there are lots of studies and products about preventing and/or treating the intra-abdominal adhesions. However, still there is no accepted method of preventing PIA (Brochhausen *et al.*, 2011; Köm, 2013; Şahbaz *et al.*, 2014). Because of the limited success and/or expensiveness of the materials used, the researchers continue to search for suitable (in terms of inexpensive, easily applicable and reachable, etc.) methods and/or products against PIA (Köm, 2013).

Some solutions such as crystalloids (e.g. sodium chloride) were evaluated as liquid barriers to prevent PIA. These liquid barriers are easily applicable. However, they failed in preventing PIA due to rapid peritoneal resorption time. The substances that can stay longer in the peritoneal cavity may be a good choice to prevent PIA (Brochhausen *et al.*, 2011). In this context, various kinds of oils with high viscosity were stated as a simple and cost-effective approach to prevent PIA (Aysan *et al.*, 2012). In the literature, commercial garlic macerated oils, a kind of viscous liquid were reported successful at different rates in preventing PIA (Şahbaz *et al.*, 2014; Ali *et al.*, 2016),

whereas some others reported vice versa (Topal *et al.*, 2019). Fresh Taşköprü Garlic macerated oil prepared in olive oil at 1,2 g/mL concentration was reported as an effective agent in preventing PIA (Çalışkan and Emin, 2022).

Medicinal plants have been widely used as a source of medicine in the treatment of various diseases since ancient times (Alan and Özen, 2021). Garlic is one of the oldest, most well-known, functional and cultivated plants all over the world (Lawson, 1998; Ferioli *et al.*, 2020). Sulfur-containing compounds are responsible for its bioactive effects, especially allicin (Zor, 2006; Yoo *et al.*, 2014). Antimicrobial drugs have been used to prevent and/or treat infections. However, drug anti-microbial resistance has been reported due to improper use of antibiotics and the infections cannot be treated effectively (Wang *et al.*, 2022). Garlic has a strong antibacterial effect due to allicin and no reports available regarding development of resistance against allicin. Because of that garlic is used widely in phytomedicine. Also, garlic has effects on increasing fibrinolysis (fibrin destruction) in connection with its antiplatelet aggregation effect (Lawson, 1998). The ability of platelets to bind to fibrinogen can be reduced by the garlic (Bhandari, 2012). Garlic macerated oil contains unique sulfur compounds such as ajoene formed by allicin transformation (Patil and Ravindra, 2008; Ferioli *et al.*, 2020). Ajoenes tend to reduce platelet aggregation (Jiang, 2019) and remain undisturbed in garlic macerated oil for a long time (Patil and Ravindra, 2008). On the other hand, small blood clots can be dissolved with garlic extracts, which activate the fibrinolytic process (Jiang, 2019). In addition to garlic, safflower oil is also known to have a reducing effect on platelet aggregation *in vitro* (Smith, 2005). In this study, it was hypothesized that a synergistic antithrombotic and fibrinolytic effect with the prepared garlic macerated oils would be valuable against PIA. This hypothesis was confirmed for the fresh garlic macerated oils (FG15, FG20).

Maceration, accepted by most of official Pharmacopoeias, is an inexpensive and easily applicable extraction method (Ferioli *et al.*, 2020). Different types of vegetable oils, such as olive and sunflower oils are used for garlic macerations (Patil and Ravindra, 2008). During current study, safflower oil was chosen to evaluate the potential effects (with/without garlic) against the PIA due to its bioactive properties and especially antiplatelet aggregation activity. According to this study's results, it is not effective to prevent PIA when used alone as group C2.

Ferioli *et al.* (2020) stated that commercial garlic extracts may contain almost no residual bioactive compound. Therefore, the importance of the analysis of garlic products before use for clinical purposes is highlighted by several researchers (Staba *et al.*, 2001; Ferioli *et al.*, 2020). Other studies on this subject were conducted using a commercial product without any laboratory analysis (Şahbaz *et al.*, 2014; Ali *et al.*, 2016; Topal *et al.*, 2019). However, in current study, commercial garlic products were not used. The different types of garlic macerated oils and even AG were produced in the laboratory. Besides, FTIR and GC-MS were used (Yoo *et al.*, 2014; Nagarajan and Kumar, 2017) to analyze the garlic-specific bioactive compounds as mentioned in the literature (Ferioli *et al.*, 2020).

In the FTIR spectra of the garlic, the positions of sulfur-containing compounds may be located in different spectral points and they are typically observed in the 500 to 1400 cm^{-1} range. The sulfur-containing compounds can also be seen in the 600 to 800 cm^{-1} range, the traces of vibrations of SH groups can be seen in this range (Nagarajan and Kumar, 2017). Diallyl-trisulfide, the most basic component of garlic essential oil, is a very important and valuable compound used as an antineoplastic, anti-inflammatory agent and platelet aggregation inhibitor (Lawson, 1998). The ratio of diallyl-sulfides is quite high in Taşköprü Garlic (Patil and Ravindra, 2008; Alshawi, 2021). In this study, it was found that garlic-specific sulfur compounds were found in certain proportions in FG macerated oils. On the other hand, garlic-specific components could not be determined in AG macerated oil. The compounds detected in the FTIR analysis may not be determined chromatographically due to very low concentration. The ratio of total trisulphites, one of the essential oil compounds obtained purely from Taşköprü garlic, was found to be 54.83% in GC-MS (Alshawi, 2021). In this study, trisulfide components were found as 1.51% and 1.98%, in FG15 and FG20, respectively. Alshawi (2021) measured the ratio of total diallyl-sulfides as 44.54%. During present study, the ratios of total diallyl-sulfides were found in promising quantity that may have strong effects against PIA.

In the food industry, the structure of vegetable oils containing linoleic acid changes due to thermal processing and leads to conversion of linoleic acid to 2,4-decadienal. This oftenly used as a flavoring additive in foods, 2,4-decadienal is a carcinogenic compound resulting from the heat treatment of linoleic acid (Schaich, 2005). Safflower oil has a high amount of linoleic acid (Smith, 2005), like garlic. Molina-Calle *et al.* (2017) stated that AG is gaining popularity. However, insufficient studies have been conducted to evaluate the effect of heat on its composition. In this study, 2,4-decadienal was detected 4.7 times higher than in FG15 and FG20. As per results obtained, it can be stated that the fermentation process caused the conversion of linoleic acid to 2,4-decadienal in AG. The changes in the serosal surface detected macroscopically and histopathologically in all of the rats of the AG20 group were directly associated with the high ratio of 2,4-decadienal in the chemical structure of the oil. These changes were intensified as time progressed post experimental procedures.

The number of rats evaluated at time points is a limiting factor in the conducted study. Although statistically significant results did not emerge ($P>0.05$) that may be due to the relatively small number of experimental rats in groups. In this context, it is very important to reveal a lot of data that would shed light on further studies on this subject.

Conclusions: According to the study results, AG15 and AG20 are not meant to prevent PIA when used intra-abdominally. Besides they may cause negative effects, especially AG20, on the injured tissue/organ surfaces due to their high ratio of 2,4-decadienal content. Although the safflower oil did not give successful results in preventing PIA when used alone, successful results were obtained in the groups used with FG. The macerated oils prepared with fresh Taşköprü Garlic in safflower oil (1.5 g/mL and

2 g/mL) were evaluated as successful agents to prevent PIA by intra-abdominal route without any irritant effect in rats at 0.1 mL dosage. In this context, FG15 and FG20 would be easily prepared in clinical and/or laboratory conditions at low cost and may be used against PIA, especially in laparoscopy without any special equipment.

Authors contributions: GÜÇ and NE designed and carried out the research (participated in the experimental study and approved the final version of the manuscript).

Declaration of competing interest: The authors declare no conflict of interest.

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