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Original Article

Effects of gelatin, pure olive oil, and hyaluronic acid on postoperative intraabdominal adhesions

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Abstract

Objective: In patients who have undergone abdominal surgery, serious adhesions are often encountered in cases of repeated abdominal surgery and problems occur in separating the intraabdominal organs. The aim of this experimental study was to investigate the effects of Gelofusine, Gelofusine plus pure olive oil, and Gelofusine plus hyaluronic acid administered to the abdomen during operations on intraabdominal adhesions that may occur after surgery.

Methods: Forty adult female Wistar albino rats weighing 200-300 g were used in the study. Rats were randomly divided into 4 separate groups with 10 animals in each group. The abdomen was entered after a 2-cm skin and subcutaneous incision along the midline of the abdomen, and then abrasion was applied to the serosa above the cecum and the right lateral peritoneal surface of the abdomen with a 1-cm child's toothbrush. In the experimental groups, Gelofusine, Gelofusine plus pure olive oil, or Gelofusine plus hyaluronic acid was applied and the abdomen was anatomically closed with 4/0 prolene threads in two layers. On the 21st day, all animals were euthanized and evaluated both macroscopically and histopathologically. In the control group, after the abdomen was opened and abrasion was applied, no liquid or other substance was administered and the abdomen was closed.

Results: No adhesions were observed macroscopically in one rat in group 1, in nine rats in group 2, in seven rats in group 3, and in four rats in group 4. Varying degrees of inflammation were found in the rats of all groups. However, fibrosis was not observed in group 2. When the groups were compared, there was a statistically significant difference between the control group and the group in which only Gelofusine (group 2) was applied ($p<0.05$).

Conclusion: Although significant results were expected in the groups in which pure olive oil and hyaluronic acid were used, only Gelofusine, which contains gelatin as an active ingredient and is used in routine human treatment, was found to be statistically and macroscopically significant in preventing intraabdominal adhesions compared to the other groups.

Keywords: Intraperitoneal adhesion, hyaluronic acid, pure olive oil, gelatin, Wistar albino rat.

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INTRODUCTION

Postoperative peritoneal adhesions that occur after abdominal surgery are one of the current problems facing surgeons (1). If abdominal surgery is performed again for patients who have previously undergone such surgery, serious adhesions are often encountered and problems are experienced in separating intraabdominal organs. In addition, the adhesions can cause difficulties in the passage of food from the intestines by causing intestinal obstructions in clinical cases (2). For this reason, patients must undergo multiple surgeries.

The aim of this experimental study was to investigate the effects of Gelofusine (3), Gelofusine plus pure olive oil (4), and Gelofusine plus hyaluronic acid (5) administered intraabdominally and postoperatively on the intraabdominal adhesions that may occur after surgery for subjects undergoing abdominal surgery. Since there is no standard treatment method in the literature for preventing intraabdominal adhesions, the study was performed on an animal model. We aimed to use these products based on examples from previous studies in the literature and to evaluate their possible superiority to each other.

MATERIALS AND METHODS

Ethical approval

Before conducting the study, permission with reference number 2019/13 was obtained from the relevant local ethics committee for animal experiments. The study was carried out in the Experimental Animals Research Center of the same university under the supervision of veterinarians. All experimental procedures were conducted in accordance with the ARRIVE guidelines and the British Animal (Scientific Procedures) Act of 1986 and related directives, EU Directive 2010/63/EU for animal experiments, and/or the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Experimental groups

Forty adult Wistar albino female rats weighing 200-300 g were used in this study. All rats were fed ad libitum before and after the experimental procedure. Standard rat food and tap water were used. All animals were housed under a 12/12-h light/dark cycle with the temperature maintained at 22 °C and ambient humidity of 60±5%. Rats were observed for at least 48 hours before inclusion in the study. Food was with held for 2 hours before the experimental procedures but water intake was not prevented. The 40 rats were randomly divided into 4 groups with 10 animals per group.

Groups

Group 1 was the control group. Group 2 received Gelofusine alone, group 3 received Gelofusine and pure olive oil, and group 4 received Gelofusine and hyaluronic acid. With the aim of adapting the treatment protocol to human settings, pure olive oil and hyaluronic acid were diluted at 1/10 with the macrolide group plasma-expanding fluid Gelofusine, which is used for stability in human hemodynamics. Gelofusine is a plasma volume complement. It contains gelatin as an active ingredient and it replaces fluid losses from the bloodstream. This intravenous plasma volume expander is a sterile liquid of the macrolide group.

Pure olive oil preparation

Pure olive oil was produced in an agricultural engineering setting with a cold-press method. The olive oil was sterilized by filtration through 0.45-nm filters into sterile centrifuge tubes. The pH value of the olive oil was the same as that of peritoneal dialysis liquid (pH 6.8).

Operative technique

Ketamine (50 mg/kg) and xylazine (10 mg/kg) anesthesia was administered intraperitoneally to all animals by the same researcher (6). After full anesthesia was achieved, the abdominal midline was shaved. In group 1, the control group, the abdomen was entered after a 2-cm skin and subcutaneous incision along the midline of the abdomen, followed by abrasion above the cecum and the right side of the peritoneal surface of the abdomen with a 1-cm child's toothbrush. The abdomen was then closed anatomically with 4/0 prolene sutures in two layers. After applying the above procedure for group 2, 10 mL of Gelofusine fluid was applied to the abdomen in contact with all intraabdominal organs. The abdomen was then closed anatomically with 4/0 prolene sutures in two layers. In group 3, the same surgical procedure was applied. Pure olive oil diluted 1/10 with 10 mL of Gelofusine liquid was then introduced into the abdomen in contact with all intraabdominal organs. The abdomen was then closed anatomically with 4/0 prolene sutures in two layers. In group 4, the same surgical procedure was applied. Hyaluronic acid diluted 1/10 with 10 mL of Gelofusine fluid was then introduced into the abdomen in contact with all intraabdominal organs. The abdomen was then closed anatomically with 4/0 prolene sutures in two layers. All animals received a single subcutaneous surgical dose of 0.01-0.05 mg/kg buprenorphine for postoperative analgesia (7). No additional doses were given due to the lack of follow-up. Antibiotics were not administered due to the sterile environment and clean surgical wounds.

Evaluation of adhesion formation

All rats were euthanized and dissected after 21 days, which is equal to 21 months of human life (one human day=30 rat days) (8). The purpose of waiting 21 days was to ensure full tissue healing (8). During the 21-day follow-up period, all rats received food and water ad libitum and no deaths occurred in any of the groups. On the 21st day, all animals were euthanized with high doses of ketamine and xylazine anesthesia intraperitoneally. The abdomen was then opened and photographed with a high-resolution camera (Figures 1 and 2). Evaluations was performed according to the presence or absence of adhesions (9).

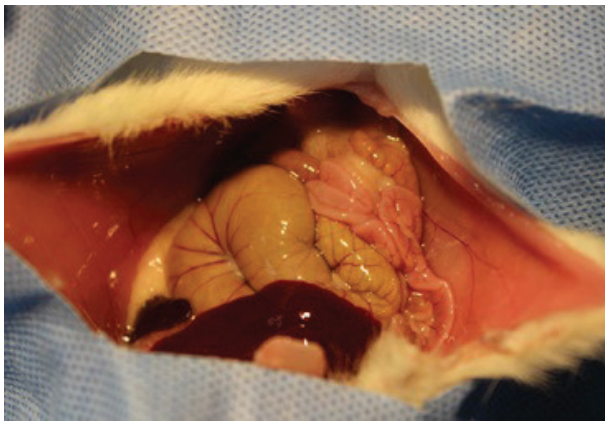

Figure 1. Intraabdominal adhesion in group 2

Figure 2. Intense intraabdominal adhesion in group 1

Histopathological evaluations

The presence of adhesions (10), level of separation, and degree of inflammation were evaluated macroscopically on the peritoneal surface. Samples including the anterior abdominal wall, peritoneum, and adherent organ(s) were obtained from all rats. All internal organs of the rats were dissected in a block. All tissues taken were fixed for 24 hours in a 10% formaldehyde solution. After fixation, a piece containing the anterior abdominal wall and tissue of 1.5 cm in length taken from the intestinal wall were blocked into two different cassettes. After the follow-up process, a sample of 4 µm in thickness was cut and stained with hematoxylin and eosin.

Statistical analysis

In power analysis performed with PASS 11 statistical software regarding sample size, it was concluded that a sample size of ten animals per group would be sufficient with an average of 1.56 unit difference

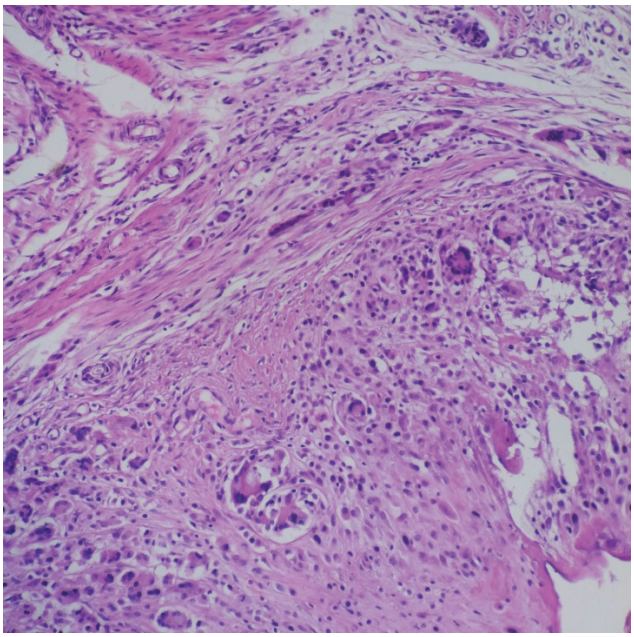

Figure 3. Widespread vascular proliferation, fibrosis, and major multinuclear inflammation in the intestinal wall of group 2

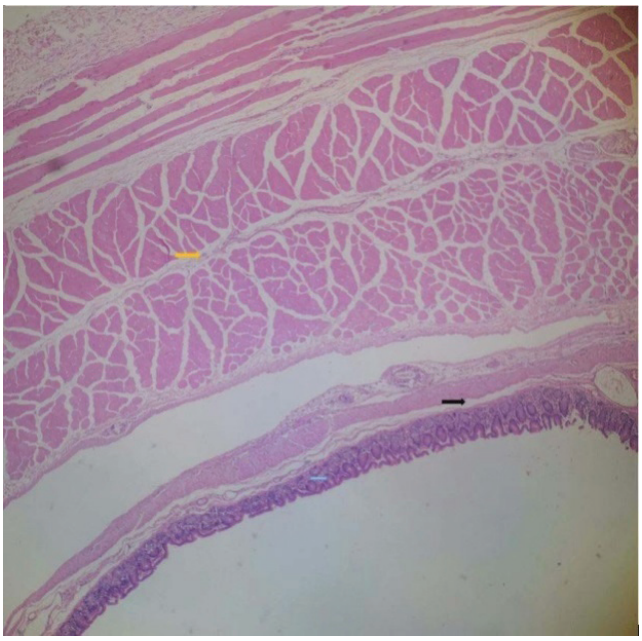

Figure 4. Widespread adhesion on the abdominal wall (yellow arrow), peritoneum (black arrow), and full-thickness bowel wall (blue arrow) in group 1

between groups, 0.8 unit standard deviation, 95% confidence interval, and ≥86% power (11). Statistical analysis was performed using SPSS 21.0 package program (SPSS Inc., Chicago, IL, USA). The chi-square test was used for the analysis of categorical variables. The Kruskal-Wallis test was used for the analysis of multiple groups and the Bonferroni-corrected Mann-Whitney U test was used in posthoc subgroup comparisons. The results were evaluated at 95% confidence intervals and at the significance level of $p<0.05$.

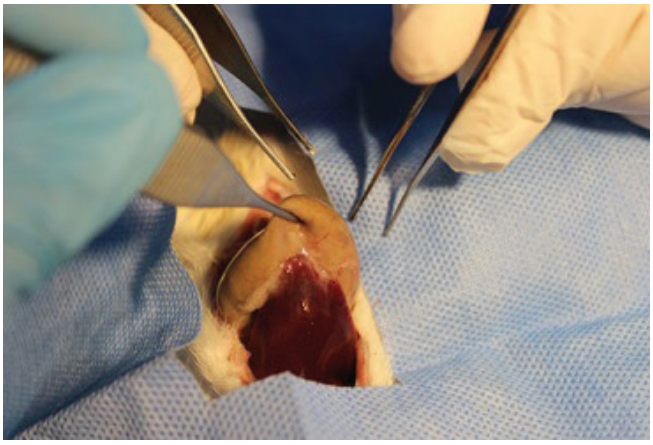

Figure 5. Intraabdominal adhesions also affected the liver in groups 3 and 4

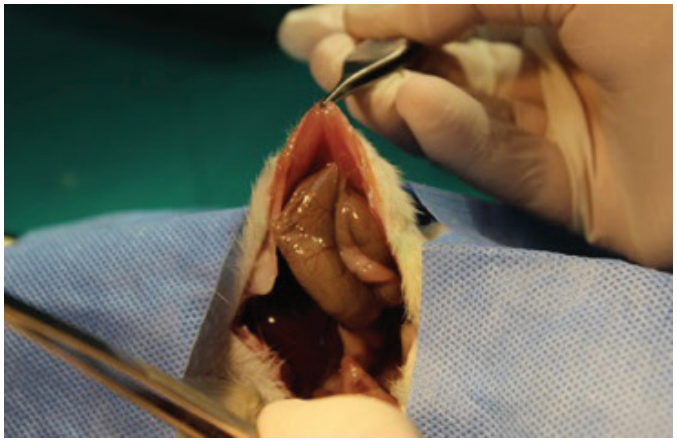

Figure 6. Intraabdominal adhesions also affected the intestines in groups 3 and 4

RESULTS

Adhesions were found in nine rats in group 1, while macroscopic adhesions were not observed in one

rat. This can be described as a good result for the control group. In this group, both inflammatory scores and fibrosis scores (12) were found to be high in histopathological examination (Figures 3-4). Adhesion was macroscopically observed in one rat in group 2, while no adhesions were observed in nine rats. Minimal fibrosis was seen in all rats of this group, while severe fibrosis was not observed. In addition, no high degrees of inflammation or Zühlke's classification (13) were detected in this group. Adhesions were macroscopically observed in seven rats in group 3, while they were not observed in three rats. In group 4, adhesions were observed in six rats, while they were not observed in four rats. However, according to the degree of fibrosis, degree of inflammation (14), and Zühlke's classification, groups 3 and 4 were at advanced stages. In groups 3 and 4, three rats with intense adhesions involving other intraabdominal organs including the liver were identified (Figures 5 and 6). There was a statistically significant difference between the groups ($p<0.03$) in terms of photographic findings and detected macroscopic materials (Table 1-5). According to the results of chi-square statistical tests between the groups, statistical significance was found between the control group and group 2 and between the control group and group 3, both macroscopically and in terms of inflammation

Table 1. Numerical values using the photographic findings

		Adhesion (-)	Adhesion (+)	Total
Groups	Group 1	1	9	10
	Group 2	8	2	10
	Group 3	7	3	10
	Group 4	4	6	10
Total		20	20	40

Table 2. Numerical values using the adhesion findings

Thin, filmy adhesions	Dense multiple visceral adhesions	Dense adhesions extending to the abdominal wall	Total
5	3	1	10
0	1	0	10
0	3	0	10
0	6	0	10
5	13	1	40

Table 3. Numerical values using the fibrosis findings

Fibrosis (-)	Minimal fibrosis	Moderate to severe fibrosis	Total
2	5	3	10
0	10	0	10
3	4	3	10
3	5	2	10
8	24	8	40

Table 4. Numerical values using the periton inflamasyon findings

		Peritonial inflammation			Total
		Inflammation (-)	Giant cell, lymphocyte and plasma cell	Giant cell, lymphocyte, plasma, eosinophil, neutrophil	
Groups	Group 1	1	6	3	10
	Group 2	3	4	3	10
	Group 3	1	6	3	10
	Group 4	2	2	6	10
Total		7	18	15	40

(p=0.04 and p=0.01, respectively). Significant results were not found in all other comparisons between the groups (p>0.05) (Table 6).

Table 5. Numerical values using the Zühlke's histopathological findings

		Loss of connective tissue, cellular richness, old and new fibrin, reticular fibrosis	Cellular connective tissue, capillary, small amount of collagen	Tight connective tissue, more vessels, elastic fibers, smooth muscle fibers	Total
Groups	Group 1	6	1	3	10
	Group 2	5	0	5	10
	Group 3	6	1	3	10
	Group 4	4	0	6	10
Total		21	2	17	40

Table 6. Statistical p values in the study

Photographic evaluation	Adhesion	Fibrosis	Peritoneal inflammation	Zühlkes's
0.002	**0.002**	0.618	0.564	0.645
0.008	0.055	0.776	1.000	1.000
0.131	0.809	0.538	0.410	0.268
0.615	0.276	1.000	0.564	0.519
0.075	**0.022**	0.618	0.256	0.661
0.189	0.189	0.776	0.410	0.268
0.005	**0.016**	0.928	0.692	0.617

The results were evaluated at 95% confidence intervals and at the significance level of p<0.05.

DISCUSSION

A review of the literature reveals a growing body of research conducted to prevent the intraabdominal adhesions that may occur after surgery, but complete standardization has not yet been achieved. Therefore, different substances and applications are being experimentally studied, but none of them have advanced beyond the experimental phase. This is likely due to the toxic effects of the substances used in excessive administration or their controversial applicability to human treatment. The importance of the present study is accordingly clear. In this study, in contrast to similar previous studies, the selected experimental substances were diluted to ratios of 1/10 with the aim of making the protocols suitable for human administration in the future.

Turgut et al. applied tamoxifen citrate, which is antineoplastic, to rats orally in 2013 and found a decrease in intraabdominal adhesions after surgery (15). However, due to the side effects of tamoxifen as an antineoplastic, together with high costs and ethical concerns, their study could not be replicated in a human setting. Giusto et al. conducted experimental studies with a pectin-honey combination in 2017, but that substance could not be used in routine practice due to costs, unavailability, and ethical concerns (16). Various other previous studies have presented meaningful results that could not be carried over into routine use (9, 17-20). Zeng et al. showed that sepra film may reduce abdominal adhesions and thus benefit patients, but it was not helpful for postoperative intestinal obstructions after open abdominal surgery. Furthermore, with sepra film, abdominal abscesses and anastomosis leakage increased (20). Altıntaş et al. achieved significant results in experiments where only pure olive oil was used (6), while Çağlayan et al. applied only hyaluronic acid (21).

In our opinion, the administration of these substances in animal models is a significant source of

controversy, as the amounts of the substances to be applied for humans are not clear. However, even if these items were to be applied in human models with established dosages, both toxicity and costs could be high depending on the amounts to be used. In our study, we found significant results among 4 groups of rats undergoing intraabdominal surgery. We diluted pure olive oil and hyaluronic acid in appropriate proportions for use in human models (i.e., 1/10 ratio) and applied them in an animal model. We anticipated significant results for the groups in which pure olive oil and hyaluronic acid were used. However, we found that Gelofusine, which contains an active gelatin ingredient and is used in routine human treatment, was statistically and macroscopically more successful in preventing intraabdominal adhesions compared to the other groups in which it was combined with pure olive oil or hyaluronic acid. This was an unexpected result. It seems that the plasma volume-enhancing Gelofusine, routinely used intravenously, may be effective in preventing intraabdominal adhesions. Cell adhesion proteins that are activated at the cellular level after any trauma, including selectins, integrins, and cadherins, settle onto the cell membrane, where they act as receptors, or they are stored in the cytoplasm (22). Proteoglycans and hyaluronic acid in extracellular matrix fibrils are relevant components in addition to structural proteins and cell adhesion molecules (23). Hyaluronic acid binds a large amount of water molecules, forming a viscous hydrated gel, which gives the connective tissue the ability to resist compression and also inhibits cell adhesion (24). In our study, we aimed to achieve less adhesion by making use of this feature of hyaluronic acid. However, there was no significant decrease in inflammation and fibrosis with the administration of that substance. Hydroxytyrosol and oleuropein, which are among the main phenolic compounds in olive oil, have a catechol (2-hydroxyphenol) structure, unlike tyrosol, and it is known that compounds with catechol groups show antioxidant activity (24). The antioxidant activity of olive oil successfully combats various oxidants and free radicals (25,26). Another study showed that olive phenols and metabolites are more effective inhibitors of lipid and protein oxidation compared to vitamins C and E; thus, polyphenols such as oleuropein, hydroxytyrosol, tyrosol, and caffeic acid in olive oil have important antioxidant and anti-inflammatory effects (25). We aimed to achieve less adhesion and inflammation by making use of this feature of olive oil. However, there was no significant decrease in inflammation and fibrosis with the administration of that substance.

CONCLUSION

In this study, Gelofusine, which contains gelatin as an active substance, was found to be more effective in preventing intraabdominal adhesions after abdominal surgery compared to pure olive oil and hyaluronic acid. Based on these results, we are thinking of continuing this research by applying Gelofusine, which is already used in human therapy, in human models for the prevention of intraabdominal adhesions. Further research on Gelofusine with larger sample sizes is needed.

Conflict of interests: The authors declare they have no conflict of interest.

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Peer-review: Externally peer-reviewed

Ethics statement: Before conducting the study, permission with reference number 2019/13 was obtained from the local ethics committee for animal experiments.

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Author contributions: Concept: Y.P., V.T.-Design: Y.P., V.T.-Supervision: Y.P.-Funding: Y.P., V.T., F.Z.Ö.-Materials: V.T., Y.P., M.E.-Data collection and/or processing: F.Z.Ö., M.E., Y.P.-Analysis and/or interpretation: F.Z.Ö., M.E., V.T.-Literature search, writing, and critical review: Y.P., V.T., M.E., F.Z.Ö.

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