Middle Black Sea Journal of Health Science / Mid Blac Sea J Health Sci

February 2022; 8(1):23-30

RESEARCH ARTICLE **DOI:** 10.19127/mbsjohs.1008275

The Role of St. John's Wort Oil (Hypericum Perforatum and Olive Oil) in the Prevention of Peritoneal Adhesion in a Rat Model: An Animal Study

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Received: 11 October 2021, Accepted: 13 December 2021, Published online: 25 February 2022

Abstract

Objective: We aimed to investigate Hypericum Perforatum (St. John's Wort Oil) and pure olive oil with respect to their effect on preventing intra-abdominal adhesions after abdominal surgery.

Methods: This was an animal study carried out by the Giresun Faculty of Medicine, Department of General Surgery between August 10, 2020, and September 10, 2020. In the study, intra-abdominal adhesion development was examined macroscopically and microscopically on the 14th day after experimental abdominal surgery in three groups [(i) Control, (ii) olive oil application, (iii) St. John's Wort oil application] each consisting of 7 female Wistar Albino rats. Majuzi classification and Zuhlke's microscopic adhesion classification was used to evaluate adhesions.

Results: Macroscopically, fibrous bands were not observed in 1 rat in the control group, 2 rats in olive oil recipients, and 1 rat in St. John's Wort oil recipients. A significant difference between the groups was identified in terms of Zuhlke's microscopic adhesion scores ($p = 0.026$). Accordingly, the adhesion level was significantly reduced in olive oil recipients compared to controls ($p = 0.002$). There was no significant difference in terms of adhesion between all groups ($p>0.05$). The Majuzi adhesion classifications in the control group, olive oil group and St. John's wort groups were also similar ($p = 0.308$).

Conclusion: In rats who underwent abdominal surgery, it was determined that intra-abdominal olive oil application reduced the level of adhesion, while St. John's Wort oil application had no effect on adhesion formation.

Keywords: Abdominal Surgery, Adhesion, St. John's Wort Oil, Olive Oil

Suggested Citation Kesicioglu T, Aydin I, Vural S,Cinar I, Gulmez M , Keskin A. The Role of St. John's Wort Oil (Hypericum Perforatum and Olive Oil in the Prevention of Peritoneal Adhesion in a Rat Model: An Animal Study.Mid Blac Sea Journal of Health Science, 2022;8(1):23-30

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INTRODUCTION

Abdominal surgery frequently results in intra-abdominal adhesions which can cause infertility and chronic pain among other effects. Difficulties in re-operations, bleeding problems, organ injuries, and other effects may also develop due to adhesion. In addition, the economic burden of hospitalizations and reoperations due to adhesions is quite high (1,2).

The main causes of adhesion formation in the abdomen are peritoneal trauma, ischemia and foreign bodies (3). Laparoscopic and minimally invasive techniques have been adopted to reduce trauma occurrence during surgical intervention. Laparoscopic surgery is associated with less intra-abdominal adhesion formation than classical open surgery; however, postoperative adhesion is still observed in patients who underwent laparoscopic methods (4). Therefore, only changing the surgical technique does not appear to be sufficient to reduce postoperative adhesions and related complications. In order to prevent adhesions, besides choosing surgical techniques that can minimize peritoneal damage, it is necessary to reduce the inflammatory response, inhibit coagulation, stimulate fibrinolysis and protect surfaces that may cause adhesion (5). Studies aimed at preventing adhesions have targeted the pathophysiological mechanism of adhesion formation. In this regard, various agents such as those suppressing inflammation and/or oxidation have been explored in addition to anti-fibrinolytic and/or anti-coagulant compounds. However, there have been no studies showing compound that can be used to prevent the development of adhesion (5-8). In addition to the antidepressant, anti-inflammatory and antibacterial properties of St. John's Wort [Hypericum Perforatum (HP)] shown in experimental studies (9-12), the positive effects of topical application of HP oil on wound healing have been shown in both animals and humans (13,14). Likewise, the anti-inflammatory, antibacterial and antioxidant properties of olive oil, which is another inexpensive and easily accessible herbal product, have been shown in various studies (15-17). Considering the mechanism of adhesion, HP and olive oil have many properties that might make them suitable for the prevention of peritoneal adhesions.

Our aim was to examine the effects of HP and pure olive oil on the formation of intra-abdominal adhesions following abdominal surgery.

METHODS

Animal Characteristics and Study Groups

In the study, 24 female Wistar Albino rats (weighing between 250 and 350 g) were included. Rats were fed with standard chow and tap water. Rats were housed in standard cages and were kept in light/dark cycles of 12 hours before and after the study (at a standard temperature of 22 °C). All animals included in the study were observed for at least 48 hours to ensure normal behavioural characteristics before inclusion into the study. Rats were weighed after 12 hours of fasting and were divided into three groups–each comprising 8 rats.

Control group: Laparotomy was performed and after palpating the ileum and cecum, the abdomen was closed.

Olive oil group: 1 ml of pure olive oil (Komili, Ayvalik, Turkey) was administered intraperitoneally after laparotomy, immediately before the abdomen was closed.

St. John's Wort group: 1 ml of pure St. John's Wort oil (Luba, Gonen, Turkey) was administered intraperitoneally after laparotomy, immediately before the abdomen was closed.

Laparotomy was performed on the rats 14 days after the procedure, and the formed adhesions were examined macroscopically and histopathologically. Since one rat in each group died after surgery, the examinations were performed on 7 rats in each of the groups.

Surgical Procedure

Experiment preparation procedures were carried out similarly in all rats. Anaesthesia was achieved by injecting 80 mg/kg ketamine HCl and 10 mg/kg xylazine HCl intraperitoneally. After shaving the abdominal skin of the subject, it was cleaned with povidone iodine and appropriate positioning was ensured in a standard heat bed used for animal surgeries. In order to provide analgesia during and after the procedure, 0.02 mg/kg fentanyl was administered subcutaneously as needed. With a 3 cm incision made from the median line, the abdomen was entered and the cecum was brought out on a wet sponge. Serosal petechiae were created on the cecum by rubbing dry sponge, and the laparotomy process was completed. In group 1, the ileum and cecum were palpated and the abdomen was closed. In group 2, laparotomy was performed and 1 ml of pure olive oil was administered intraperitoneally before the abdomen was closed. In group 3, laparotomy was performed and 1 ml of St. John's Wort oil was administered intraperitoneally before the abdomen was closed. During abdominal wall closure, the fascia was sutured with 2/0 polydioxanone suture and the skin was sutured with 3/0 silk. A single dose of cefazolin (20 mg/kg) was administered intramuscularly for postoperative antibiotic prophylaxis. In the postoperative period, normal oral feeding was achieved by all rats. The rats were sacrificed on the 14th postoperative day and evaluated macroscopically and microscopically in terms of adhesion development.

Macroscopic Evaluation

Postoperative intraperitoneal adhesions were ascertained using the Majuzi classification [Grade 0: lack of adhesion, Grade 1: small irregular adhesions, Grade 2: medium density but easily separable, Grade 3: Dense but irregular and cannot be separated easily, Grade 4: Almost complete, regular, cannot be separated easily]. An experienced surgeon blinded to groups evaluated macroscopic adhesion.

Histopathological Evaluation

Samples were obtained from fibrotic bands located between the peritoneum and cecum. A pathologist blinded to rat groups performed the histopathological examination. After the cecum samples taken from the subjects had been fixed in 10% formaldehyde, they were made into paraffin blocks and cut into 5 µm sections. Finally, staining was performed with haematoxylin and eosin, and histopathological examination was performed under light microscopy. Interstitial fibrosis and inflammatory cell reaction were evaluated in the examination. Zuhlke's microscopic adhesion classification was used to evaluate adhesions.

Zuhlke's microscopic adhesion classification (Figure 1):

- Grade 0: No adhesion, no interstitial reaction.
- Grade 1: Weak connective tissue, rare cell, old and new fibrin, thin reticulin fibrils.

- Grade 2: Connective tissue with few cells and capillaries.
- Grade 3: Thicker connective tissue, dense cells, denser and thicker-walled vessels, rare elastic and smooth muscle fibres, rare collagen fibres.
- Grade 4: Thick or nodular granulation tissue, dense collagen fibre and smooth muscle fibres.

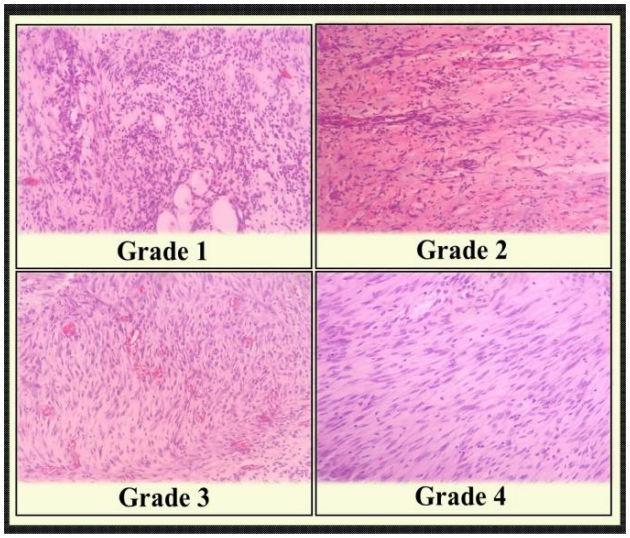

Figure 1. Zuhlke's microscopic adhesion classification histological image samples (H&E stain 20x100)

Statistical analysis

Statistics and analyses of results were performed on the 21.0 version of SPSS (IBM, Armonk, NY, USA). For the normality check, the Kolmogorov-Smirnov test was utilized with Lilliefors correction. Data are given as median (minimum - maximum) for continuous variables, and as frequency for categorical variables. Between-groups comparison of these variables were performed by analyzing differences between measurements with the Kruskal-Wallis test. The Bonferroni correction was used for pairwise comparisons. P values of <0.05 were defined to demonstrate statistical significance in all analyses.

RESULTS

Since one rat in each group died after surgery, no examination could be performed in terms of adhesion in these rats. Macroscopically, fibrous bands were not observed in 1 rat in the control group, 2 rats in olive oil recipients, and 1 rat in St. John's Wort oil recipients. A significant difference between the groups in terms of Zuhlke's microscopic adhesion scores was found (p = 0.026). Accordingly, the levels of adhesion were significantly lower in the olive oil group compared controls (p = 0.002). Adhesion degrees were similar between all groups (p>0.05). (Figure 2).

The comparison of Majuzi adhesion classification findings between the control, olive oil and St. John's Wort groups also yielded non-significant results (p = 0.308) (Table 1).

Table 1. Adhesion grade with regard to group

	Control (n = 7)	Olive oil (n = 7)	St. John's Wort oil (n = 7)	*p-value*
Zuhlke's microscopic adhesion classification	3 (2 – 4)[a]	1 (0 – 2)[b]	2 (1 – 3)[ab]	0.003
0	0 (0%)	2 (28.6%)	0 (0%)	
1	0 (0%)	2 (28.6%)	1 (14.3%)	
2	1 (14.3%)	3 (42.9%)	3 (42.9%)	
3	4 (57.1%)	0 (0%)	3 (37.5%)	
4	2 (28.6%)	0 (0%)	0 (0%)	
Majuzi adhesion classification	2 (1 – 3)	2 (0 – 3)	2 (2 – 3)	0.310
0	0 (0%)	1 (14.3%)	0 (0%)	
1	2 (28.6%)	0 (0%)	0 (0%)	
2	4 (57.1%)	3 (42.9%)	4 (57.1%)	
3	1 (14.3%)	3 (42.9%)	3 (42.9%)	

Data are given as median (minimum – maximum) for continuous variables and as frequency (percentage) for categorical variables.
[a,b]: Same letters denote the lack of statistically significant difference between groups

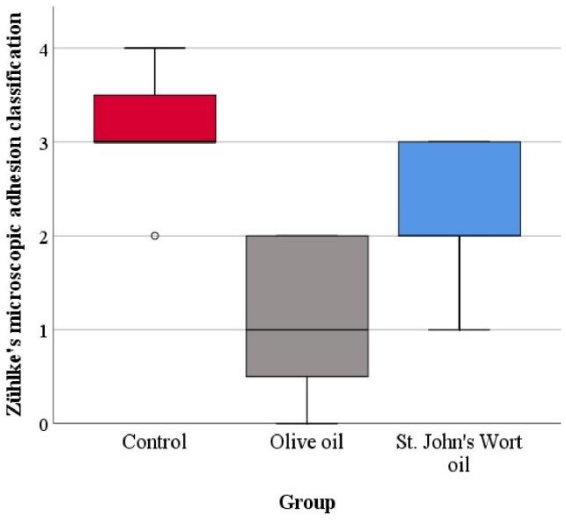

Figure 2. Zuhlke's microscopic adhesion classification with regard to group

DISCUSSION

Advances in the understanding of intra-abdominal adhesion mechanisms has led to use of many substances aimed at preventing adhesion formation. The pathophysiology of adhesion includes tissue damage and the subsequent inflammatory response, and there are many studies that have sought to prevent adhesion formation with agents targeting these factors (18,19).

Many studies have reported that HP has anti-inflammatory, antioxidant and antibacterial properties (9-12). These properties of HP are attributed to its various components, including quercetin, I3, II8-biapigenin and hypericin (20,21). In our study, considering that the development of adhesion after abdominal surgery was due to similar pathophysiological mechanisms, intra-abdominal HP was applied to rats that underwent abdominal irritation and the development of adhesion was examined. However, in the analyses performed, it was determined that the level of inflammation and adhesion formation in the St. John's Wort oil group was indifferent when compared with the controls and olive oil recipients group. Similar to our study, in an animal study investigating olive oil, St. John's Wort oil and control groups, Hızlı et al. reported that adhesion, inflammation and fibrosis levels were not different in the St. John's Wort oil group compared to other groups. Consistent with our study, the adhesion level in the olive oil group was lower than that of the control group, while it was found to be similar to the St. John's Wort oil group (22). Although other studies drew attention to St. John's Wort oil, in our study, it was observed that although the adhesion in St. John's Wort oil recipients was relatively lower compared to controls, statistical significance was not present. This may be due to the low number of animals per group in our study.

In various studies examining the properties of pure olive oil, it has been determined that it has anti-inflammatory, antibacterial, antioxidant and antineoplastic properties (15-17). It has also been shown that it can improve wound formation with its various properties (23). Currently, it is being suggested that olive oil can prevent adhesion with respect to its hydroflotation feature resulting from its high viscosity value (24). In addition, it has been stated that squalene, beta sitosterol, erythrodiol and some other components in olive oil have anti-inflammatory activity (25). As a result, we observed that adhesion formation occurred at a lower frequency in rats that had received pure olive oil application. When studies focusing on adhesion investigations are examined, various outcomes can be observed throughout the literature. In an animal study conducted with a model similar to ours, Ural et al. (24) applied 5 ml of pure olive oil to rats, for which

they created an abdominal surgery model, and reported that no adhesion formation could be identified in the intra-abdominal region 30 days after the operation. The authors attributed this finding to anti-inflammatory effects and properties that support tissue regeneration. Hızlı et al. (22) also stated that they found lower adhesion olive oil recipients relative to controls. The results of these studies are in support of our findings. Although no examination was made on olive oil content and mechanism of action in our study, it was thought that the anti-inflammatory components shown in other studies may have an adhesion-reducing effect (24,25). In experimental models with more animals, it may be feasible to suggest long-term follow-up of rats with intra-abdominal pure olive oil application, thereby enabling longer-term comparisons and elucidation of possible side effects, which may, in turn, lead to studies examining the utility of olive oil application for adhesion prevention in humans.

The primary limitations of our study are animal count per group and the short postoperative follow-up period in terms of adhesion development. In addition, one animal died in each surgical group before measurements could be performed. The cause of death in these animals may be due to the application of foreign agents, or may be a direct result of surgical stress, and therefore, such effects should be investigated in larger series.

CONCLUSION

In this animal study, in which the effects of St. John's Wort oil and olive oil were examined with regard to adhesion formation after intra-abdominal surgery, it was determined that St. John's Wort oil (HP) did not alleviate adhesion, whereas olive oil had protective properties and reduced the degree of adhesion. In future studies, the effect of olive oil and St. John's Wort oil on adhesion can be revealed in more detail by including more animals, applying different doses, and examining longer-term results.

Ethics Committee Approval: Ethics committee approval was obtained from Giresun University Rectorate, Faculty of Health Sciences Dean's Office, Animal Experiments Local Ethics Committee Directorate (Number: 12763492-770-E.32531, Date:24.07.2020).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept: T.K., I.A., S.V.; Design: T.K., I.C., M.G.; Literature Search: T.K., I.A., A.K.; Data Collection and Processing: S.V., M.G.; Analysis or Interpretation: T.K., A.K.; Writing: T.K., I.A., S.V.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure*:* The authors declared that this study hasn't received no financial support.

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