Effect of *Hypericum perforatum* on intraperitoneal adhesion formation in rats

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Abstract

Introduction: The aim of this study was to evaluate the efficacy of *Hypericum perforatum* for prevention of adhesion formation in rats.
Material and methods: Twenty-four female wistar rats underwent left uterine horn adhesion model. Rats were randomised into 4 groups. Group 1 (Control): Closure of abdominal incision without any agent administration. Group 2: Closure of incision after administration of intraperitoneal (*i.p.*) Ringer's lactate solution. Group 3: Closure of incision after administration of *i.p.* olive oil (diluent of *H. perforatum*). Group 4: *Hypericum perforatum* extract (Ecodab®) was administered *i.p.* before the closure of incision. Fourteen days later, relaparatomy was performed and surgical adhesion scores, inflammation and fibrosis scores were noted. Groups were compared according to these scores.
Results: There was statistical significant difference between ringer's lactate group and olive oil group according to surgical adhesion score ($p = 0.009$). However, groups were not different according to inflammation and fibrosis scores ($p > 0.05$).
Conclusions: Despite antiinflammatory, antioxidants and antimicrobial properties of *H. perforatum*, our results revealed no positive effect of *H. perforatum* on the prevention of intraperitoneal adhesion formation.

Key words: *Hypericum perforatum*, intraperitoneal adhesion, prevention, rat, St. John's wort.

Introduction

Postoperative adhesions are an important problem and develop after almost all laparotomies [1]. Adhesions may cause severe clinical problems such as bowel obstruction, infertility and chronic pelvic pain [2–4].

Previous studies have evaluated different agents to reduce the incidence of intraperitoneal adhesions, including removal of fibrinous exudates by peritoneal lavage, use of proteolytic enzymes, anticoagulants, steroids, antihistamines, cytotoxic agents and use of substances such as olive oil or liquid paraffin [3, 5, 6]. However, the benefits of such methods in relation to intraperitoneal adhesions remain unclear. Moreover, despite strong efforts to reduce postoperative adhesions, 50% of patients still

develop significant adhesions [7–11]. Therefore, new approaches to this problem are warranted.

The sequence of adhesion formation has been reported as follows: tissue ischemia, inflammation, fibrin deposition, fibrin organization, collagen formation, and maturation with the formation of adhesions [1, 3, 5, 12]. In an attempt to reduce the inflammatory reaction at the site of the peritoneal trauma, recent studies have focused on the use of anti-inflammatory drugs [3]. One anti-inflammatory agent which has not been studied for anti-adhesive efficacy previously is *Hypericum perforatum*.

Hypericum perforatum, commonly known as St. John's wort, is a yellow-flowering perennial herb grown in temperate and subtropical climates that has a long history of use as a medicinal plant for treating wounds and skin ailments, nerve problems, muscle pain, and mood disorders such as depression and anxiety [13–15]. *Hypericum perforatum* was first described by Robson in 1968 [16]. Many studies revealed this extract's positive effect on wound healing due to its anti-inflammatory, antimicrobial and antioxidant effects [17, 18]. From this point of view, we decided to evaluate whether *H. perforatum* extract has a positive effect on postoperative adhesion formation in a rat model.

Material and methods

Twenty-four 4-5 month-old female Wistar rats weighing 250 ±20 g were housed in a climate-controlled (relative humidity of 40 ±5% and temperature of 21°C to 24°C) animal care facility, with a 12-hour light/dark cycle. Before and after surgical procedures, the animals were provided with standard rat chow and water, ad libitum. After adaptation, the animals were randomly assigned to 4 different groups, each consisting of 6 rats.

Anesthesia was induced by injection of ketamine (45 mg/kg *i.m*. of Ketalar; Eczacibasi, Istanbul, Turkey) and xylazine (5 mg/kg) anesthesia. The surgical procedures were performed under sterile conditions. Before the surgery, gloves were washed extensively with saline to remove any particles of powder. All operations were performed by the same author who was blinded to the treatment group. The rats were randomly assigned and not sequentially operated on in order to minimize bias. The operation was limited to 10 min for each rat and antibiotic prophylaxis was not given.

A 3-cm vertical midline incision was performed and both uterine horns were exposed. Punctate serosal hemorrhages were generated by scraping with a No. 15 scalpel blade until petechial bleeding emerged at the abdominal sidewall and antimesenteric surface of the left uterine horn to create adhesions. The abdominal incision was closed in two layers using a simple interrupted 4-0 polyglactin 910 suture for the peritoneum–fascia and for the skin.

Prior to closure of the abdominal incision, the following agents were given in the abdominal cavity.

Group 1 (Control): Closure of abdominal incision without any agent administration. Group 2 (Ringer's lactate): 2 ml/rat intraperitoneal (*i.p*.) of Ringer's lactate solution was administered before closure of the incision. Group 3 (Olive oil): 2 ml/rat of olive oil was administered to the rats *i.p*. (diluent of *H. perforatum*). Group 4 (*H. perforatum*): 2 ml/rat *H. perforatum* extract (Ecodab®) was administered *i.p*.

The second laparotomy with a U-shaped incision was performed 14 days after the first surgery. Adhesions were scored according to the clinical adhesion scoring system of Leach *et al*. [19] by the same author who performed the first laparotomy. The author was blind to which group was being evaluated. A total score of 10 was possible. Adhesions to the uterine horn defect were scored as follows: 0 = no uterine adhesion; 1 = 1–25% involvement; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100%. Adhesions were further characterized on gross examination for severity as follows: 0 = no adhesions; 1 = filmy avascular; 2 = vascular or opaque; 3 = cohesive attachment of uterine horns to each other or other abdominal structures. The degree of adhesion formation was evaluated with the following adhesion scores: 0 = no adhesions; 1 = if the adhesion was separated from tissue with gentle traction; 2 = requiring moderate traction; and 3 = requiring sharp dissection.

Histopathological examination was performed by one investigator. Adhesion-carrying tissues were excised en-bloc and fixed in a 10% buffered formaldehyde solution. The tissue samples were embedded in paraffin wax, cut into 5 µm sections and stained with hematoxylin-eosin and Masson's trichrome. Inflammation was scored as follows: 0 – no inflammation; 1– presence of giant cells, occasional lymphocytes and plasma cells; 2 – presence of giant cells, plasma cells, eosinophils and neutrophils; and 3 – presence of many inflammatory cells and microabscesses. The amount of fibrosis was scored as: 0 = no fibrosis; 1 = minimal, loose; 2 = moderate; and 3 = florid dense.

The experimental procedures in the present study were approved by the Gazi University School of Medicine Ethics Committee (No: 11.001) and supported by the Scientific Research Fund of Fatih University under the project number P53011001_G 1471.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS for Windows, Version 15.0, Chicago, Illinois). Kruskal-Wallis and Mann-Whitney U tests corrected with Bonferroni were used. A value of $p < 0.05$ was regarded as significant.

Table I. Median surgical, inflammation and fibrosis score of groups

Variables	Control	Ringer	Olive oil	SJW	Value of *p*
Surgical score	8 (2)	8 (1.5)	5.5 (3.25)	4 (1.5)	0.009†
Inflammation score	1 (1)	2 (1)	1.5 (1.25)	2 (0.25)	0.035
Fibrosis score	1 (1.25)	1 (1.25)	1.5 (1.25)	2 (1.25)	0.221

SJW – St. John's wort (Hypericum perforatum), p < 0.0125 regarded as statistically significant (Kruskal-Wallis and Mann-Whitney U tests corrected with Bonferroni were used). †There is a statistically significant difference between olive oil and Ringer group

Table II. Comparison of the adhesion, inflammation and fibrosis score of groups

Variables	Values of *p*		
	Surgical adhesion grade	Inflammation	Fibrosis
Sham vs. ringer	0.310	0.026	0.240
Sham vs. olive oil	0.026	0.589	0.310
Sham vs. SJW	0.065	0.041	0.065
Ringer vs. olive oil	0.009	0.180	0.937
Ringer vs. SJW	0.065	0.699	0.485
Olive oil vs. SJW	0.818	0.240	0.394

SJW – St. John's wort (Hypericum perforatum)

Results

Median surgical adhesion scores of the groups are presented in Table I. Adhesion score was lower in the olive oil group when compared to the Ringer's lactate group and control group and there was a statistically significant difference between the Ringer's lactate group and olive oil group ($p = 0.009$). Rats treated with *H. perforatum* had lower surgical adhesion scores when compared to the olive oil group; however, the difference was not significant ($p = 0.8$) (Table II). Differences between each group according to surgical adhesion score are shown in detail in Table II. Appearance of intra-abdominal adhesion is shown in Figure 1.

Median inflammation and fibrosis scores of each group are presented in Table I. The histological appearances of fibrosis and inflammation are shown in Figure 2. The olive oil group had a lower inflammation score when compared to Ringer and *H. perforatum* groups but the difference between groups was not statistically significant (Table II). Similarly, no difference was detected between groups according to the fibrosis score.

Discussion

In the present study, *H. perforatum* was evaluated for efficacy in the prevention of postoperative adhesion formation in a rat adhesion model. The results showed that administration of *H. perforatum* was not effective to prevent or reduce intra-abdominal adhesions.

Postoperative adhesions, formed after abdominal surgery, develop in up to 95% of patients and can lead to serious complications including small bowel obstruction, infertility, dyspareunia, difficulty with future operations, and possible chronic pelvic pain [1, 20, 21].

Intra-abdominal adhesion formation is initiated by the increase in vascular permeability and secretion of fibrin-rich exudate which are triggered by peritoneal injury. Under normal conditions, these

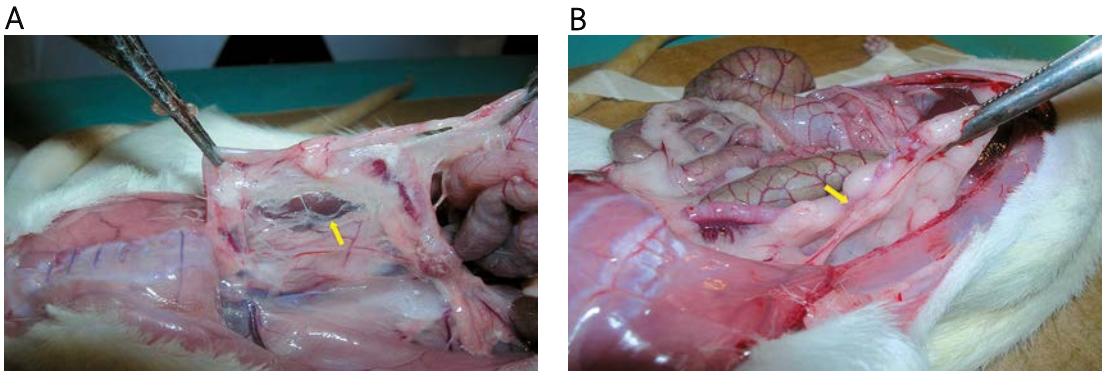

Figure 1. The appearance of dense intra-abdominal adhesions in rat

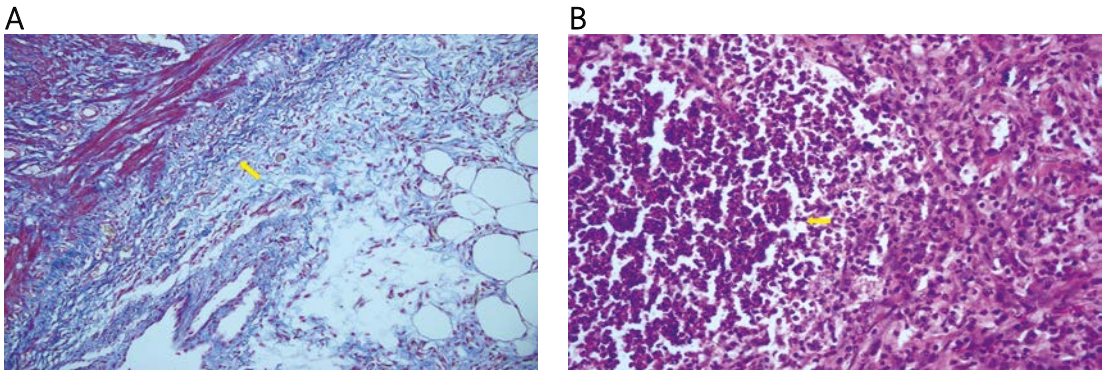

Figure 2. Histologic appearance of fibrosis (**A**) (Masson trichrome stain 200×) and inflammation (**B**) (hematoxylin and eosin stain 400×)

bands are resolved by fibrinolysis. However, under ischemic or inflammatory conditions, the peritoneal fibrinolytic system is suppressed and these bands are infiltrated with inflammatory cells and fibroblasts, which create dense adhesions. Studies that aim to prevent adhesions have focused on the prevention of various steps of this physiopathological process. Antiinflammatory agents, antioxidants, anticoagulants, fibrinolytics and bioabsorbable physical barriers have been used in this regard [3, 22]. Despite positive reports, there is still no consensus about this issue [23, 24].

Experimental studies showed that *H. perforatum* has antiinflammatory, antioxidant and antimicrobial properties [17, 18, 25, 26]. As the physiopathological process of adhesion formation is considered, *H. perforatum* has many characteristics which make it potentially suitable in the prevention of peritoneal adhesions. From this point of view, we hypothesized that *H. perforatum* may prevent or reduce postoperative intra-abdominal adhesion formation.

Hypericum perforatum has been used as a medicinal plant for more than 2000 years. The anti-inflammatory effect of *H. perforatum* was attributed to quercetin, I3,II8-biapigenin and hypericin inside the extract [27]. *In vitro* hypericin has been shown to inhibit tumor necrosis factor α-induced activation of the transcription factor NF-κB that is involved in the immunological and inflammatory response [28]. It was reported that the anti-inflammatory effect of *H. perforatum* extract appeared to be, at least partly, a consequence of inhibition of NF-κB by hypericin. Also hypericin significantly and dose-dependently inhibited the release of arachidonic acid and, as a result, of leukotriene B4, and strongly inhibited interleukin 1α (IL-1α), possibly by inhibiting protein kinase C [29, 30]. Interleukin 1α is well known as a potent factor in mediating inflammation and fever.

Hypericin also possesses antioxidant activity, acts as a free-radical scavenger [31], inhibits the formation of interleukin (IL)-1a and IL-12 and inhibits the release of arachidonic acid from phospholipids and its metabolism through 5- and 12-lipoxygenase pathways [29].

Moreover, in 2000, Schempp *et al*. reported the immunomodulatory effect of a *H. perforatum* ointment, which reduced stimulation of T lymphocytes by epidermal cells after application to skin [32].

Some animal studies have reported a benefit from Ringer's lactate in preventing adhesions [33, 34]. It has been stated that Ringer's lactate prevents adhesion formation by mechanical cleaning of blood clots at the surgical site [35]. Also it is supposed that the presence of a high volume of the solution in the abdominal cavity separates raw peritoneal surfaces and thus prevents adhesion formation [36]. On the other hand, in two clinical studies, the effect of dextran solution on postoperative adhesion formation was compared with that of Ringer's lactate solution [36, 37]. In both studies, no beneficial effect of Ringer's lactate was found, and many of the patients treated with Ringer's lactate had more adhesions at the time of second examination laparoscopy than before the initial procedure. In the present study, the peritoneal adhesions treated with Ringer's lactate also did not show a significant decrease when compared to the control group.

To the best our knowledge there is no study which has evaluated the efficacy of *H. perforatum* for prevention of adhesion formation. From this point of view the present study is the first study in which the anti-adhesive role of *H. perforatum* has been studied. Despite anti-inflammatory, antioxidant and antimicrobial properties of *H. perforatum*, no positive effect of *H. perforatum* on the prevention of intraperitoneal adhesion formation was found in the present study. This finding suggests that other mechanisms of adhesion formation beside ischemic or inflammatory conditions may be responsible for adhesion formation after surgery. More detailed studies are needed on this topic and future studies should clarify the exact pathophysiological mechanisms of adhesion formation to develop safe and effective agents to achieve maximum efficacy in the prevention of adhesions.

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