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Anti-adhesive effects of argan oil on postoperative peritoneal adhesions

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Abstract

The aim of this study is to reveal effect of Argan oil on postoperative peritoneal adhesion. Twenty-four Wistar albino rats were divided into three groups. After laparotomy was carried out intraperitoneally, 0,9% NaCl and 3 ml Argan Oil applied to saline and Argan oil groups, respectively. Four subjects in each groups were sacrificed at postoperative day 3 and 7. Macroscopic adhesions and microscopic cellular reactions, such as giant cell, lymphocyte/plasmocyte, neutrophil and histiocyte, were assessed and hydroxyproline levels were measured in all three groups. Adhesion and fibrosis scores were lower both 3rd and 7th days in Argan oil, but only lower fibrosis scores were statistically significant ($p<0,05$). Except giant cell 3th day scores; Argan oil had lowest neutrophil, lymphocyte, plasmocyte, and histiyocyte scores. Both 3rd and 7th days scores of neutrophil, lymphocyte, plasmocyte, but only 7th days scores of histiocyte reaction were statistically significant ($p<0,05$). However; Argan oil had highest hydroxyproline levels and the difference were not statistically significant both 3rd and 7th days ($p>0,05$). Argan oil reduced the postoperative peritoneal adhesions initially seperation the damaged tissues, subsequent effects of fatty acids and tocopherols on inflammation, plasminogen activation and matrix metalloproteinase steps of adhesion formation.

Keywords: Peritoneum, postoperative, adhesion formation, fatty acids, argan oil

Introduction

Postoperative peritoneal adhesion (PPA) is one of the major complications of abdominopelvic surgery and occurs 70 to 93 % of the patients and 33 % caused to twice or more readmissions to hospital [1,2]. PPA are associated with intestinal obstructions, chronic pain, secondary infertility, re-operation requirments and high cost [1,2]. PPA occurs after contact of damaged peritoneal or visceral surfaces initally. Adhesion formation is started as local inflammatory reaction with fibrinous exudate and fibrin formation [3,4]. Fibrin deposition and degradation balance are important for determining normal peritoneal healing or formation of adhesion [5].

Various kind of agents have been performed for prevention of PPA. Barriers, surgical or non invasive strategies, cellular strategies, drugs and agents have been used at clinical or experimental studies [6]. Fatty acids contents such as soybean oil, ClinOleic, canola oil, omega 3 fatty acids and olive oil were studied for prevention of PPA experimentally in literature [7-11].

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Argan oil is a natural oil which has been widely used in Morocco. Argan oil is rich in unsaturated fatty acids (80%) especially oleic (44,8%) and linoleic acids (33,7%) and contains antioxidant combinations, such as tocopherols (especially γ-tocopherol) [12]. Argan oil has been reported usage of burn and surgical wound treatments [13,14]. We considered that argan oil which is cheap, easy accessible and nautral, can be used for prevention of peritoneal adhesions due to intraabdominal surgical procedures. The aim of this study is to reveal the effect of Argan oil on experimental postoperative peritoneal adhesions.

Material and Method

Animals

This study was performed at the Experimental Animal Laboratory of Uludağ University after obtaining the approval of Animal Ethics Committee. All protocols were in accordance with the regulations concerning the care and use of laboratory animals as in the Universal Declaration on Animal Welfare.

Twenty four Wistar albino out-bred female rats (mean weight, 250±30 g, mean age 7 months) divided into 3 groups and were kept in standard rat cages, each containing maximum five rats, 12 hr light / 12 hr dark cycle, and at stable temperature between 19-22 C0. They were fed with standard rat pellet and tap water [9,10].

Experimental Model

After a 12-hour starvation, isoflurane inhalation anestesia were used for anesthesia. Rats were laid on supine position and their extremities were fixed to the operating table using medical plaster. After abdominal skin shaving, antisepsis was provided by povidone iodine solution. All applications which could lead to adhesion were avoided [9,10].

After midline incision of 2 cm-in length, the cecum was pulled out of the abdomen, and 1 cm-in length insicion was performed. Stool was seen in the side of cecal wound and sutured by 3/0 silk one by one. Thereafter, cecum was returned into the normal position.

For control group (C) nothing, for saline group (S) 3 ml of 0,9 % saline solution, and for Argan oil group (AO) 3 ml of argan oil were instilled intraperitoneally. Midline incision was closed with 4/0 polypropylene running sutures. 100 mg/kg of paracetamol was injected subcutaneously for analgesia. The rats were allowed normal feeding, 6th hour postoperatively [9,10].

Macroscopic Assessment

Four rats from each group were sacrificed at 3rd and 7th days postoperatively with 100-150 mg/kg high dose sodium thiopental. The peritoneal cavity was entered via a "reverse U" incision. Subsequently, anterior abdominal wall, peritoneal cavity, small bowels and cecum were examined carefully and assessed according to the scoring scale [15] (Table 1). After macroscopic evaluation, a cecal segment of 2 cm-in length with neighboring mesenteric root were resected for both histopathologic and biochemical examination [9,10]. Sacrificed animals were putted to the Uludağ University Experimental Animal Laboratory's medical waste and the study was completed.

Table 1. Macroscopical and histopathological assesment

Scores	Macroscopical Evaluation	Histopathological Evaluation
0	No Adhesions	None
1	Thin and narrow, easily separable adhesions	Rare
2	Thick adhesions limited to one area	Mild
3	Thick and wide adhesions	Severe
4	Thick and wide adhesions between organs and abdominal wall	------

Histopathologic Assessment

Resected cecal specimens were fixed in 10% formole solution. After dehydration, they were embedded into the paraffin at tissue follow-up equipment. Two slices of 3 μ in thickness were prepared by microtome from each specimen [9,10].

First slice was stained with hematoxylene and eosin for assessing giant cell, lymphocyte/ plasmocyte, neutrophil, and histiocyte reaction. Second one was stained with Masom-tricom for fibrosis assessment according to standard staining protocols [9,10].Giant cell, lymphocyte / plasmocyte, neutrophil, histiocyte reaction and fibrosis were scored from zero to 3 [16] (Table 1). Microscopic assessment was realized by light microscopy under x 100, and x 200 magnification. The pathologist in the study group was blinded during the interpretation of the results [9,10].

Biochemical assessment

Cecal samples for biochemical assessment were put into dry tubes and taken to the biochemistry laboratory. All tissues were homogenized by 70 mL tissue in 1 mL %0,9 NaCl solution. Adding equal volume of HCl, homogenized tissues were incubated at 95 C0 water bath for 24 hours [9,10].

Acetate citrate buffer (pH:6,5), chloramines T reactive and erlich reactive were prepared freshly as study reactives. Hydroxyproline study standards were prepared. At the end of the study, absorbent value from samples and standards were quantified by measuring at spectrophotometer [9,10].

Statistical Analysis

All statistical analyses were performed by a statistical software package (SPSS 16.0). Numerical data were expressed as mean and standard derivation. Kruskal-Wallis test was used for statistical analysis of giant cell, lymphocyte/plasmocyte, neutrophil, histiocyte reaction, fibrosis scores as their values were nonparametric, and the number of subjects in each group was lower than 30. Statistical significance of hydroxiproline levels were assessed by using ANOVA test because all values obtained from three groups were parametric [9,10]. Results were expressed with a confidence interval of 95% and p values below 0,05 were considered as statistically significant.

Results

No surgical complication or mortality was seen during study. The results of study are shown at Table 2. Adhesion scores were noted as 2,5±0,57, 2,0±0,0 and 1,25±0,5 at 3rd day, 2,75±0,95, 1,75±0,5 and 1,25±0,95 at 7th day in Control, Saline and Argan Oil groups, respectively. Third and 7th day scores were lower in Argan Oil group but not statistically significant (p=0,28, p=0,91 respectively).

Giant cell reaction was not seen at 3rd day in Control group (none). The difference between 3rd and 7th day scores at giant cell reaction were highest in Control group but not statistically significant (p=0,25, p=0,258 respectively). Control and Saline groups had similiar and higher neutrophil scores in both at 3rd and 7th days and the difference were statistically significant (p=0,05 both days). Argan Oil group had lower lymphocyte and plasmocyte scores at both 3rd and 7th days and the difference were statistically significant (p=0,02, p=0,05 at lymphocyte, p=0,01, p=0,009 at plasmocyte respectively). Histiocyte reactions were lower in Argan Oil group at both 3rd and 7 th day but only 7th days scores statistically significant (p=0,3, p=0,03 respectivley). Fibrosis scores were lower in Argan Oil group both at 3rd and 7th day and statistically significant (p=0,03, p=0,01 respectively).

Hidroxyproline levels were measured as 0,239±0,125 mg proline/g protein in Control group, 0,13±0,056 mg proline/g protein in Saline group and, 0,381±0,259 mg proline/g protein in Argan Oil group at 3rd day. Hidroxyproline levels were measured as 0,154±0,032 mg proline/g protein in Control group, 0,128±0,043 mg proline/g protein in Saline group, 0,166±0,071 mg proline/g protein in Argan Oil group at 7th day. 7th day scores were lower in all groups but the differences were not statistically significant (p=0,146, p=0,567 respectively).

Table 2. Results of macroscopic, histopathologic and biochemistry assessment. (Mean ± Standard Derivation, SD), * mg proline/g protein

		Adhesion Score	Giant Cell	Neutrophil	Lymphocyte	Plasmocyte	Histiocyte	Fibrosis	Hydroxyproline*
Control	**3th**	2,5±0,57	0,0±0,0	3,0±0,0	1,5±0,57	1,5±0,57	1,5±0,57	2,0±1,15	0,239±0,125
	7th	2,75±0,95	1,75±1,5	3,0±0,0	2,5±0,57	2,5±0,57	2,5±0,57	2,75±0,5	0,154±0,032
Saline	**3th**	2,0±0,0	1,75±0,5	3,0±0,0	2,75±0,5	2,5±0,57	1,75±0,5	1,5±1,29	0,13±0,056
	7th	1,75±0,5	2,25±0,95	3,0±0,0	2,75±0,5	3,0±0,0	2,5±010	3,0±0,0	0,128±0,043
Argan Oil	**3th**	1,25±0,5	0, 5±1,0	1,5±0,57	1,25±0,5	1,0±0,0	1,0±0,81	0,0±0,0	0,381±0,259
	7th	1,25±0,95	1,0±0,0	1,75±0,5	1,5±0,57	1,0±0,0	1,0±0,0	1,25±0,5	0,166±0,071

Discussion

Postoperative peritoneal adhesions are the major complications that caused to intestinal obstruction, secondary infertility, chronic pelvic pain, requirement of reoperations after abdominopelvic surgery [17]. Contact of damaged intraperitoneal surfaces due to surgery is important step for starting adhesion formation. Increased vascular permeability followed by an exudation of inflammatory cells and formation of fibrin matrix are the subsequent steps of adhesion formation. Connections consist between damaged tissue or peritoneum with modeling fibrin bands. Destruction of this fibrin bands by fibrinolitic system is provided peritoneal healing or reduction on fibrinolitic system provide persisting of fibrin band [18-20].

Carboxymethil cellulose + hyaluronic ascid, oxide regenere cellulose, %4 icodextrin are the barriers which provides adhesion prevention with mechanical seperation and approved by American Food and Drug Administiration. Drugs or the other agents have prevention effects on various steps of adhesion formation in literature [6].

Argan oil is a vegetable oil which has wound healing effect, hepatoprotection effect and preventive effect on hypercholesterolemia and atherosclerosis traditionally. These effects are related with consomption of fatty acids and antioxidant. The essential fatty acids are oleic acid with 43-49% and linoleic acid with 29-36%. Schottenol and spinasterol are the essential sterols with 44–49% and 34–44% respectively and γ-tocopherol is the esential tocopherols with 81–92% [13,14,21]. We considered that these fatty acids and tocopherols could reduce postoperative adhesion formation.

Adhesion scores, fibrosis and hidroxyproline levels are the sign of adhesion formation and severity. In recent study; adhesion scores were lower and similar both 3rd and 7th days in Argan oil (not statistically significant). Fibrosis was not occured during 3rd day and fibrosis scores were significantly lower both 3rd and 7th days in Argan oil. However hidroxyproline levels were higher with decreasing gradually both 3rd and 7th days in Argan oil. Argan oil reduced adhesion formation and severity during first 7 days due to following reasons;

Gel or liquid barriers have been used for seperation of damaged surfaces during the first 5-7 days that required for peritoneal re-epithelialization [6]. Argan oil reduced adhesion formation with its liquid form like as soybean oil, olive oil, omega 3 fatty acids [7-11].

Inflammation is important for peritoneal adhesion formation. Additionaly the anti-inflammatory effect of fatty acids, Jiang et al reported that γ-tocopherol inhibits proinflammatory PGE2 and LTB4, decreases TNF-alpha, and γ-tocopherol shows anti-inflammatory effect [7-11,22].

Except giant cell, Argan oil reduced the inflammatory cells during first seven days that is important time for occuring adhesion formation postoperatively. These inflammatory cells; (1) Neutrophils are the initial inflammatory cells that occurs early period of peritoneal healing. Decreasing neutrophils and phagocytosis reduced peritoneal adhesions. (2) Histiocytes are the important cells of chronic inflammation and major component of monocyte–phagocytic system. Macrophages have an important role on adhesion formation related with inflammation and plasminogen activators. Decreased macrophages are associated with reduced adhesion formation. (3) Plasmocyte and lymphocytes play a crucial role in chronic inflammation which trigger the subsequent adhesion maintenance [9,10].

Giant cells are seen in granulamatous inflammation that is different patern of chronic inflammatory reaction and generally occurs due to foreign bodies [9,10]. Lowest score of control group first three days was associated with absence of the preventive applications, but chronic inflammation occured subseguently. However Argan oil demonstrated low foreign body reaction during seven days.

Tissue plasminogen activators convert the plasminogen to plasmin and plasmin ensures degradetion of fibrin at fibrinolitic system [2]. Higazzi et al. reported that oleic acid stimulates plasminogen activation and modulates the fibrinolytic and autolytic activities of plasmin [23].

Matrix metalloproteinases (MMP) are a large enzyme family that degrade various components of Extracellular Matrix, and inhibited by Tissue-derived Inhibitors of Matrix metalloproteinase (TIMP). Increasing of MMP and decreasing of TIMP were reduced peritoneal adhesions [19]. Oleic acid modulates the MMP activity and expression [24].

Conclusion

In conclusion our study demonstrated that Argan oil reduced the postoperative peritoneal adhesions initialy seperation of damaged tissues, subsequent effect of fatty acids and tocopherols on inflammation, plasminogen activation and matrix metalloproteinase steps of adhesion formation. Long term studies are needed to evaluate the further effects of Argan oil on postoperative peritoneal adhesions.

Competing interests
The authors declare that they have no competing interest

Financial Disclosure
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