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The Effects of Nigella Sativa Oil to Prevent Postoperative Adhesions İn Experimental Rat Model

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Original Research Article

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Abstract: Postoperative intraabdominal adhesion (PIA) formation is an important cause of morbidity and mortality in patients undergoing abdominal surgery. This experimental study aims to evaluate the effects of Nigella sativa oil (NSO) on fibrinolytic system, while reducing PIA formation. A total of 16 Wistar Albino rats, each weighting 200 to 250 g, were used in this study. All rats were divided into two separate groups, as Group 1 (control group; n=8) and Group 2 (experimental group; n=8). An experimental adhesion model was formed in which Group 1 rats were administered with 1 mL isotonic saline intraabdominally, while Group 2 rats were administered with 1 mL NSO. The rats were sacrificed on postoperative day 10.Tissue plasminogen activator (tPA) concentration in the blood and peritoneal fluid was measured using the enzyme-linked immunosorbent assay (ELISA), and adhesion formation was evaluated using macroscopic and microscopic examinations. Group 1 had significantly lower peritoneal fluid TPA concentrations (0.34±0.08 ng/mL vs1.53±1.44 ng/mL; $p<0.01$) and significantly lower serum concentrations for this compound (1.12±0.55 ng/mL vs 2.25±0.91 ng/mL; $p<0.01$). Based on macroscopic examinations, Group 2 showed significantly lower adhesion size and extension ($p<0.01$). Histopathological examinations revealed that Group 2 showed lower collagen levels, fibroblastic activity, and increased vascularity, indicating a significant difference between the groups ($p<0.01$). This study shows that intraperitoneal NSO administration may reduce PIA by increasing the fibrinolytic activity in the rat model of intraabdominal adhesion formation.
Keywords: Intraabdominal adhesions, nigella sativa oil, tissue plasminogen activator.

INTRODUCTION

Intraabdominal adhesions are defined as pathological fibrotic bands formed in the peritoneal cavity [1]. Intraabdominal adhesions increase the length of hospital stay and related with surgical morbidity and mortality[2]. Mortality rate ranges from 6 to 13% in patients with mechanical intestinal obstruction due to adhesions [3, 4]. They also increase the rates of re-hospitalization and re-operation.

Although exact physiopathology of adhesion formation has not been fully understood, trauma and ischemia are thought to initiate adhesion formation by inducing the release of tissue factors [5]. During the healing process, fibroblasts locally proliferate due to insufficient fibrinolytic activity and lead to permanent adhesions. Experimental studies have demonstrated a direct relationship between decreased fibrinolytic activity and adhesion formation [5]. Activation of fibrinolytic system induces conversion of plasminogen to plasmin. Plasmin is effective in converting fibrin to fibrin breakdown products. TPA and urokinase are plasminogen activators (uPA). The former is largely responsible for plasminogen activation in the peritoneal cavity [6]. Either of these substances is released from endothelial cells, mesothelial cells and macrophages [6]. As a result, drugs targeting this pathway can be used to prevent PIA formation. The resulting effect as a widely accepted mechanism is formation of fibrous adhesions due to tissue ischemia and decreased tPA concentrations following surgery [7].

Nigella sativa is an herbaceous annual plant which belongs to the Ranunculaceae family. It is cultivated in Mediterranean countries, and in Pakistan and India [8]. It is also known as the black seed and it is natural medicine used in the treatment of many diseases [8]. Nigella sativa possesses many biological activities, such as immunomodulation, anti-inflammatory effects, analgesic, antiviral, and antineoplastic effects [8, 9]. The Nigella sativa oil (NSO) exerts unique biological activities through more than 30 volatile (18.4 to 24% thymoquinone and 46% monoterpenes) and non-volatile oils [10, 11]. Its seed contains bioactive compounds such as essential fatty acids, sterols and tocols, and are used as antioxidant agents [12, 13].

Currently, most of the adhesion preventers show their effects through their anti-inflammatory and antioxidant effects. In this study, we aimed to evaluate the effects of NSO on fibrinolytic activity in reducing PIA.

MATERIALS AND METHODS

Ethics Committee Approval

Ethics Committee approval for this experimental study was obtained from R.T. Marmara University Animal Experiments Local Ethics Committee (97.2013.mar; Istanbul, Turkey). The subjects were supplied from R.T. Marmara University Experimental Animals Application and Research Center and the study was conducted in the laboratory of R.T. Marmara University Experimental Animals Application and Research Center.

Experimental Groups

Sixteen Wistar-Albino rats weighting 200-250 grams were used in the study. The rats were divided into two groups each comprising 8 rats. Group 1: Control Group, Group 2: Intraperitonal NSO group. Rats were kept in the same laboratory environment for one week before the experiment. Rats were maintained on a cycle of 12 hours of light and 12 hours of darkness at room temperature and maximum four rats were placed in the standard cages. In the preoperative and postoperative period, rats were fed standard chow and water.

Surgical Technique

General anesthesia was performed with intraperitoneal administration of ketamine 100 mg/kg (Ketalar, Parke Davis, and Istanbul, Turkey).After anesthesia; the rats were placed in supine position. Extremities were immobilized with a wound plaster. Anterior abdominal wall was shaved and the area was cleaned with povidone iodine solution (Betadine, Kurtsan, and Istanbul, Turkey). Standard instruments were used in surgical procedures. Peritoneal cavity was entered through a 3-cm midline incision. Group 1 (Control Group): After stretching 0.5 cm of peritoneum with a clamp and ligating its base with 4/0 silk sutures (Dogsan, Istanbul, Turkey), peritoneal adhesion knots of the inner abdominal wall were formed with three on each side, then 1 ml of sterile serum physiologic solution was instilled into the abdominal cavity and the incision was closed with 3/0 polypropylene continuous sutures (Prolene, Dogsan, Istanbul, Turkey). Group 2 (Nigella Sativa Group): After stretching 0.5 cm of peritoneum with a clamp and ligating its base with 4/0 silk sutures (Dogsan, Istanbul, Turkey), peritoneal adhesion knots of the inner abdominal wall were formed with three on each side, then sterile NSO solution was instilled into the abdominal cavity and the incision was closed with 3/0 polypropylene continuous sutures. All rats were administered analgesics after the procedure and left for recovery from anesthesia. Six hours after operation, rats were fed ad libitum standard chow and tap water.

Sacrifice and Assessment

In the postoperative follow-up period, no rats were lost due to surgery or anesthesia complications. At day 10, all rats received intraperitoneal ketamine 100 mg/kg to induce general anesthesia. Skin was stripped off the fascia to avoid injury to the implantation line. Abdominal wall was opened caudally with “inverse U” incision without damaging adhesion formation. After withdrawing a 1 cc of peritoneal fluid for peritoneal fluid tPA measurement, intra-abdominal adhesions were evaluated quantitatively according to the Nair’s Macroscopic Classification [14]. The evaluation was performed by two separate investigators in a double-blind fashion as per the classification system they were previously taught. Following macroscopic examination, adhesions were removed together with the affected organs in rats that developed adhesions, while peritoneal ischemia areas were excised pathological examination as to include all layers except the skin in rats that did not develop adhesions. Then, pathology specimens were fixed in containers containing 10% buffered formalin solution. This was followed by sacrifice by draining off the blood from the heart while the rats were under general anesthesia and blood samples were centrifuged at 1000 g for 15 minutes. Plasma samples were transferred into the eppendorf tubes and stored at -20°C for the analysis of tPA concentrations.The sample were handled in cold chain tPA concentrations were determined using micro ELISA method (Rat tPA ELISA Kit, Innovative Research, Novi, USA) as per the instruction in the Biochemistry Laboratory of Bezmialem University.

Histopathological examinations were performed in the Pathology Laboratory of R.T Medeniyet University Goztepe Education and Research Hospital. The specimens were followed with classical laboratory methods and embedded in paraffin blocks. Five-micrometer-thick sections were placed on the slides. The sections were stained with hematoxylin-eosin and examined under the light microscope. The examining pathologist was kept blind to the group of the specimen. After histopathological examination, specimens were classified according to the Zülke’s microscopic classification [15].

Statistical analysis

Statistical analysis was performed using the NCSS version 2007 software (NCSS LLC., Kaysville, UT, USA). Descriptive statics were expressed in mean, standard deviation, median, frequency, and ratio. The Mann-Whitney U test was used to compare normally distributed variables between the groups. A p value of <0.05 was considered statistically significant with 95% confidence interval (CI).

RESULTS

Hematological findings

Statistically significant difference was found between the groups with respect to peritoneal fluid TPA concentrations ($p<0.05$). The measurements of subjects in the NSO group were significantly higher. The mean peritoneal TPA concentration was 1.53±1.44 ng/ml in the NSO group and 0.34±0.08 ng/ml in the control group (Table 1).

Blood tPA concentrations were significantly differ between the two groups ($p<0.05$). The measurements of subjects in the NSO group were significantly higher. The mean blood TPA concentration was 2.25±0.91 ng/ml in the NSO group and 1.12±0.55 ng/ml in the control group (Table 1).

Adhesion scores

There were significant differences between the groups with respect to size and extensiveness of the adhesions ($p<0.01$). The median value of adhesions in the intraperitoneal NSO group was significantly lower compared to the control group.

Histopathologic Findings

There were significant differences between the groups with respect to the amount of collagen fibers, vascularization, and fibroblastic activity ($p<0.01$). The median value in the NSO group was significantly lower compared to the control group (Table 2). The microscopic appreances of specimens were shown in.

Table-1: Comparison of tPA Concentrations in the Blood and Intraperitoneal Fluid between the Control and Study Groups

		tPA		[a]p
		Nigella Sativa (n=8)	Control (n=8)	
Peritoneal Measurements	mean±SD	1.53±1.44	0.34±0.08	0.009**
	Min-Max (Median)	0.35-3.55 (0.6)	0.21-0.49 (0.3)	
Blood Values	mean±SD	2.25±0.91	1.12±0.55	0.021*
	Min-Max (Median)	0.80-3.42 (2.3)	0.34-2.01 (1.1)	

[a]Mann-Whitney U Test **p<0.01 *p<0.01

Table-2: Comparison of the Amount of Collagen Fibers, Vascularization and Number of Fibroblasts in Microscopic Examination

		Microscopic Classification		[a]p
		Nigella Sativa (n=8)	Control (n=8)	
Amount of Collagen Fiber	+	0 (0)	4 (50.0)	
	++	2 (25.0)	4 (50.0)	
	+++	6 (75.0)	0	
Min-Max (Median)		2-3 (3)	1-2 (1.5)	0.002*
Vascularization	+	0 (0)	5 (62.5)	
	++	3 (37.5)	3 (37.5)	
	+++	5 (62.5)	0 (0)	
Min-Max (Median)		2-3 (3)	1-2 (1)	0.002*
Number of Fibroblasts	+	0 (0)	5 (62.5)	
	++	3 (37.5)	3 (37.5)	
	+++	5 (62.5)	0 (0)	
Min-Max (Median)		2-3 (3)	1-2 (1)	0.002*

[a]Mann-Whitney U Test **p<0.01

DISCUSSION

Mechanical obstruction associated with PIA can occur after surgery in 4% of all laparotomies [16-18). Approximately 70% of all small bowel obstructions are caused by PIA [18]. Following surgery due to PIA, 12-20% of patients require reoperation [19].

Injury to the peritoneum due to traumatic causes triggers a cascade of events that initiate adhesion formation [20]. Injury to the peritoneal mesothelial cell surface results in increased leukotriene B4 and prostaglandin E2 levels in the peritoneal fluid and inhibition of TPA activation [21]. Increased levels of leukotriene B4 and prostaglandin E2 induces adhesiogenesis, and inhibition of TPA activity decreases fibrin breakdown and triggers PIA formation [21].

Peritoneal injury activates coagulation cascade that results in thromboplastin release and fibrin production. If fibrin breakdown is insufficient, this provides the environment for PIA formation. If there is

excessive fibrin production, this exceeds the capacity of peritoneal plasmin to degrade fibrin and PIA is formed [20, 22, 23].

There are studies reporting that heparinization of the blood in peritoneal cavity following peritoneal injury can prevent PIA formation [22]. Particularly, insufficient fibrinolytic activity and abnormal peritoneal healing may result in PIA formation. Fibrinolysis may not be completed in the presence of excessive thromboplastin-derived fibrin production and inhibition of tPA activity [20, 23]. If enzymes released from the leukocytes upon inhibition of fibrinolytic activity fail to degrade fibrin exudate, fibrinous adhesions further progress to form a fibrotic band. Migration of fibroblasts and collagen accumulation enlarges these fibrin bands to form fibrous adhesions with regression of capillaries and accumulation of fibroblasts and permanent adhesions are formed [24-26]. The imbalance between TPA and its inhibitor (PAI-1) increases adhesion formation [27-29]. Enteral form of n-acetylcysteine has been shown to increase blood tPA levels in adhesion model and thereby decrease PAI formation by increasing fibrinolytic activity [30]. The studies to date have shown that nigella sativa reduced PIA formation; however, there is no sufficient data on its effects on fibrinolytic mechanism. In the present study, we examined TPA concentrations in the peritoneal fluid and blood in order to evaluate fibrinolytic effects of NSO. Blood and peritoneal fluid TPA concentrations were significantly higher in the NSO, compared to the control group ($p<0.01$).

Inflammatory mediators may play an important role in PIA formation [31]. Some studies have shown that anti-inflammatory drugs reduced PIA formation in mice, rats and rabbits [32-34]. Nigella sativa oil possesses anti-oxidant, analgesic, antiviral, antineoplastic, anti-inflammatory, and immunomodulatory activities [8, 11]. The studies found that N. sativa eliminate adverse effects of free oxygen radicals through its anti-inflammatory and anti-oxidant properties and thereby exert cytoprotective and anti-apoptotic effects [8, 35-36]. Nigella sativa oil was shown to reduce apoptosis in rat model of necrotizing enterocolitis [37]. In addition, NSO was shown to have positive effects in preventing PIA formation and in addition this effect could be mediated by reducing apoptosis and collage formation in the damaged tissues [38]. Instillation of NSO into the abdominal cavity following cecal abrasion was shown to reduce inflammation, fibrosis, and vascularization at the microscopic level [39]. Similar to other studies, the present study found a statistically significant decrease in microscopically evaluated amount of collagen fibers, vascularization and fibroblastic activity in the NSO group when compared to the control group.

In conclusion, intraperitoneal administration of NSO is thought to increase fibrinolytic activity and decrease PIA formation by increasing TPA concentrations in the blood and peritoneal fluid. Therefore NSO can be beneficial to prevent intraabdominal adhesion formation. However, large-scale studies are needed to confirm these findings.

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REFERENCES

1. Hellebrekers BW, Trimbos-Kemper TC, Trimbos JB, Emeis JJ, Kooistra T. Use of fibrinolytic agents in the prevention of postoperative adhesion formation. Fertil Steril. 2000; 74: 203-12.
2. Chu DI, Lim R, Heydrick S, Gainsbury ML, Abdou R, D'addese L, Reed KL, Stucchi AF, Becker JM. N-acetyl-l-cysteine decreases intra-abdominal adhesion formation through the upregulation of peritoneal fibrinolytic activity and antioxidant defenses. Surgery. 2011 Jun 1;149(6):801-12.
3. DeCherney AH; diZerega GS. Clinical problem of intraperitoneal postsurgical adhesion formation following general surgery and the use of adhesion prevention barriers. Surg Clin North Am. 1997; 77: 671-688.
4. Muller SA, Treutner KH, Tietze L, Anurov M, Titkova S, Polivoda M. J Surg Res. 2001; 96: 68-74.
5. Holmdahl L, Ericsson E, Ericsson BI, Risberg B. Depression of peritoneal fibrinolysis during operation is a local response to trauma. Surgery. 1998; 13: 539-44.
6. Arung W, Meurisse M, Detry O. Postoperative peritoneal adhesions. World J Gastroenterol. 2011 November 7; 17(41): 4545-4553.
7. Ten Broek RP, Issa Y, van Santbrink EJ, Bouvy ND, Kruitwagen RF, Jeekel J, Bakkum EA, Rovers MM, van Goor H. Burden of adhesions in abdominal and pelvic surgery: systematic review and met-analysis. Bmj. 2013 Oct 3;347:f5588.
8. Ali BH, Blunden G. Pharmacological and toxicological properties of Nigella sativa. Phytother Res. 2003; 17: 299–305.
9. Pahdhye S, Benarjee S, Ahmad A, Mohammad R, Sarkar FH. From here to eternity-the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer Ther. 2008; 6: 495–510.
10. Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med. 1995; 61: 33–36.
11. Kanter M, Coskun O, Budancamanak M. Hepatoprotective effects of Nigella sativa L and

Urtica dioica L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World J Gastroenterol. 2005; 11: 6684–6688.
12. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytotherapy research. 2000 Aug;14(5):323-8.
13. Piras A, Rosa A, Marongiu B, Porcedda S, Falconieri D, Dessì MA, Ozcelik B, Koca U. Chemical composition and in vitro bioactivity of the volatile and fixed oils of Nigella sativa L. extracted by supercritical carbon dioxide. Industrial Crops and Products. 2013 Apr 1;46:317-23.
14. Nair SK, Bhat IK, Aurora AL. Role of proteolytic enzyme in the prevention of postoperative intraperitoneal adhesions. Arch Surg. 1974;108(6):849–853.
15. Zühlke HV, Lorenz EM, Straub EM, Savvas V. Pathophysiology and classification of adhesions. Langenbecks Arch Chir Suppl II Verh Dtsch Ges Chir. 1990:1009–1016.
16. Wilson MS, Hawkswell J, McCloy RF. Natural history of adhesional small bowel obstruction: counting the cost. Br J Surg. 1998;85:1294-8.
17. Monk BJ, Berman ML, Montz FJ. Adhesions after extensive gynecologic surgery: clinical significance, etiology, and prevention. Am J Obstet Gynecol. 1994; 170: 1396-1403.
18. Ellis H. The clinical significance of adhesions: focus on intestinal obstruction. Eur J Surg. 1997; 22: 5-9.
19. Menzies D. Postoperative adhesions: their treatment and relevance in clinical practice. Ann R Coll Surg Engl. 1993; 75:147-53.
20. Gomel V, Urman B, Gürgan T. Pathophysiology of adhesion formation and strategies for prevention. J ReprodMed 1996; 41: 35-41.
21. Drollette CM, Badawy SZA. Pathophysiology of pelvic adhesions: modern trends in preventing infertility. J Reprod Med. 1992;37:107-121.
22. Ryan GB, Grobéty J, Majno G. Postoperative peritoneal adhesions: a study of the mechanisms. The American journal of pathology. 1971 Oct;65(1):117.
23. Zerega GS. Biochemical events in peritoneal tissue repair. Eur J Surg. 1997;577:10-16.
24. Buckman RF, Woods M, Sargent L. A unifying Pathogenetic Mechanism in the etiology of intraperitoneal adhesions. J Surg Res. 1996; 20: 1-5.
25. Knightly J, Agostine D, Cliffton E. The effect of fibrinolysin and heparin on the formation of peritoneal adhesions. Surgery. 2002; 52: 250-8.
26. Holmdahl L, Risberg B, Beck DE, Burns JW, Chegini N, Ellis H. Adhesions: pathogenesis and prevention-panel discussion and summary. The European journal of surgery. Supplement.:= Acta chirurgica. Supplement. 1997(577):56-62.
27. Liakakos T, Thomakos N, Fine PM, Dervenis C, Young RL. Peritoneal adhesions: etiology, pathophysiology, and clinical significance. Recent advances in prevention and management. Dig Surg. 2001; 18: 260 –273.
28. Diamond MP, Decherney AH. Pathogenesis of adhesion formation/ reformation: application to reproductive pelvic surgery. Microsurgery. 1987; 8: 103–107.
29. DiZerega GS, Rodgers KE. The Peritoneum. New York: Springer- Verlag; 1992.
30. Chu DI, Lim R, Heydrick S, Gainsbury ML, Abdou R, D'addese L, Reed KL, Stucchi AF, Becker JM. N-acetyl-l-cysteine decreases intra-abdominal adhesion formation through the upregulation of peritoneal fibrinolytic activity and antioxidant defenses. Surgery. 2011 Jun 1;149(6):801-12.
31. Golan A, Bernstein T, Wexler S, Neuman M, Bukovsky I, David MP. The effect of prostaglandins and aspirin—an inhibitor of prostaglandin synthesis—on adhesion formation in rats. Human Reproduction. 1991 Feb 1;6(2):251-4.
32. Ustün C, Koçak I, Akpolat I. Effects of Seprafilm (sodium hyaluranate-based bio resorbable), Sepracoat (0.4% hyaluronicacid), and Ringer's lactate on the prevention of postsurgical adhesion formation in rat models. J Obstet Gynaecol. 2000; 20: 78–80.
33. Hellebrekers BW, Trimbos-Kemper GC, vanBlitterswijk CA, Bakkum EA, Trimbos JB. Effects of five different barrier materials on postsurgical adhesion formation in the rat. Hum Reprod. 2000; 15: 1358–1363.
34. Greene AK, Alwayn IP, Nose V, Flynn E, Sampson D, Zurakowski D, Folkman J, Puder M. Prevention of intra-abdominal adhesions using the antiangiogenic COX-2 inhibitor celecoxib. Annals of surgery. 2005 Jul;242(1):140.
35. Terzi A, Coban S, Yildiz F, Ates M, Bitiren M, Taskin A, Aksoy N. Protective effects of Nigella sativa on intestinal ischemia-reperfusion injury in rats. Journal of Investigative Surgery. 2010 Feb 1;23(1):21-7.
36. Bayrak O, Bavbek N, Karatas OF, Bayrak R, Catal F, Cimentepe E, Akbas A, Yildirim E, Unal D, Akcay A. Nigella sativa protects against ischaemia/reperfusion injury in rat kidneys. Nephrology Dialysis Transplantation. 2008 Jan 22;23(7):2206-12.
37. Tayman C, Cekmez F, Kafa IM, Canpolat FE, Cetinkaya M, Uysal S, Tunc T, Sarıcı SU. Beneficial effects of Nigella sativa oil on intestinal damage in necrotizing enterocolitis. Journal of Investigative Surgery. 2012 Sep 25;25(5):286-94.
38. Karatas A, Ozlu T, Ozyalvacli G, Tosun M, Cetinkaya A, Donmez ME, Turker A, Bayrakdar H. Intraperitoneal Nigella sativa for prevention of postoperative intra-abdominal adhesions in rats.

Journal of Investigative Surgery. 2014 Dec 1;27(6):319-26.
39. Sahbaz A, Ersan F, Aydin S. Effect of Nigella sativa oil on postoperative peritoneal adhesion formation. J Obstet Gynaecol Res. 2014; 40(2): 532–7.