Assessment of postoperative adhesion formation in a rat cecum model using different techniques

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Abstract: Adhesion-related complications after abdominal surgery bring out momentous morbidity and costs. Outcomes from animal experiments investigating prevention of adhesions are limited due to lack of consistency in existing animal models. Different intraperitoneal adhesion models were compared the interobserver variability was evaluated to seek for best model. Forty male SD rats weighting 250-300g were included and assigned randomly to four groups with diverse techniques, (A) postoperative adhesion cecum rat model abraded with sterile rasp; (B) postoperative adhesion cecum rat model abraded with sterile dry gauze; (C) postoperative adhesion cecum rat model abraded with sterile blade; (D) postoperative adhesion cecum rat model abraded with vascular clamp. Macroscopic adhesion scores were evaluated by Bigatti scoring system, and the incidence of adhesion were surveyed on the 7th day after the surgery. The results showed that four techniques currently used Bigatti adhesion scoring system are subjective, the sterile rasp is the most consistent and reproducible tool to establish an intraperitoneal adhesion model which is helpful for related studies and the development of new substances for adhesion prevention in the future.

Keywords: Adhesions; peritoneal; rat model.

INTRODUCTION

Postoperative peritoneal adhesions develop between deperitonealized surfaces of abdominal organs, mesenteries and the abdominal wall; the most common site of adhesion formation is between the greater omentum and the anterior abdominal wall (Ouaïssi *et al.,* 2012). It is a common but serious complication caused by peritoneum damage after abdominal-pelvic surgery. Over 95 percent of the previous literature have reported that patients would develop adhesions after surgery (Parker *et al.,* 2007; Cheong *et al.,* 2001; Cassidy *et al.,* 2014). According to available data, peritoneal adhesions form in 93-100% of cases after laparotomy for upper abdominal surgery in adults (Nunobe *et al.,* 2008). Many patients, if not most, have increased the risk of long term sequela, including obstipation (Schnuriger *et al.,* 2011), ileus, chronic pelvic and abdominal pain (ten Broek *et al.,* 2013), sterility, and difficult secondary surgery (Brüggmann *et al.,* 2010; Ergul and Korukluoglu, 2008). Moreover, reoperations which probably prolong surgical time, increase iatrogenic intestinal lesion and recurrence of small intestine obstruction are very complicated (Rajab *et al.,* 2010). Postoperative intra-abdominal adhesions also exert a tremendous impact on health care expenses (Ward and Panitch, 2011).

Peritoneal adhesions develop within the first 5 to 7d after injury to the peritoneum, and can be caused by mechanical trauma, foreign body reactions, ischemia and radiation (Lin *et al.,* 2014). The process of postoperative adhesion formation is complex and involves inflammatory reaction, mesothelial cells, an imbalance in fibrin formation and fibrinolysis and a remodeling and phagocytic phase. Therefore, they are an object of frustration for veterinary and human surgeons, which has stimulated research into products and methods to prevent their formation (Alonso Jde *et al.,* 2014).

Many experiments on the prevention of peritoneal adhesion have been carried out to prevent or treat adhesions (Karacam *et al.,* 2011; Hong *et al.,* 2011). Lots of anti-adhesive agents, for example, anti-inflammatory agents, nanofibrous membranes, physical barriers including liquid and solid barriers, fibrinolytic agents and antibiotics have been applied to prevent the formation of postsurgical intra-abdominal adhesions in a variety of animal models (Dinarvand *et al.,* 2012; Park *et al.,* 2007; Zhang *et al.,* 2011; Sahbaz *et al.,* 2014). However, there is not a gold pathological standard for the animal model of intra-abdominal adhesion. We found a multitude of abdominal adhesion modeling methods using tools like scalpel blade (Belluco *et al.,* 2001; Lai *et al.,* 2003), dry sterile gauze (Zhang *et al.,* 2011; Ozturk *et al.,* 2010; Panahi *et al.,* 2012) and forceps hemostatic (Ryan *et al.,* 1971) on the ascending colon (Yarema and Mogamedov, 2003), cecum (Cashman *et al.,* 2011) and stomach (Li *et al.,* 2013). Horses (Gorvy *et al.,* 2008; Parker *et al.,* 1989), pigs (Lauder *et al.,* 2012; Alkhamesi and Schlachta, 2013), rabbits (Pascual *et al.,* 2013; Hermans *et al.,* 2012), rats (Lauder *et al.,* 2011), mices (Burnett *et*

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al., 2006; Condon *et al.,* 2006) and sheep (Zinther *et al.,* 2010) were used as modeling animals. Adhesion models include abdominal sidewall defect (Parsaei *et al.,* 2013), cecal abrasion and uterine horn injuries (Karaca *et al.,* 2013; Batukan *et al.,* 2007), but the results have been inconsistent. Experiments in our laboratory obtained varied outcomes when explored adhesion models (cecal abrasion model), and there is little information available in literature about comparing different cecum models in rats of peritoneal adhesion. The aim of this study is to evaluate the quantity and quality of adhesion creation in four rat cecum models in order to screen the comparatively objective peritoneal adhesion cecum model.

MATERIAL AND METHODS

The animal experiments reported in this investigation complied with guidelines of Institutional Animal Care and Use Committee at the First Clinical Medical College, Nanjing University of Chinese Medicine. All protocols were performed adhere to the Animal Welfare Act. Sprague-Dawley. The rats were kept on a 12-hour light-dark schedule and enabled free access to standard rat pellet and tap water. All the rats were placed in a cage for fasting (liquids only) one day before the surgery. The male SD rats were anesthetized with 10% chloral hydrate (300mg/kg) and all the surgical procedures were conducted under semi sterile condition. After the abdomen was shaved and the skin was disinfected with 2% iodine, 1 cm incision was performed in the middle of the abdomen. After abdominal closure, the rats received 3 ml penicillin (Streptocillin Vet®; Boehringer Ingelheim; Denmark) i.m. The injections were repeated for the next 3 days. After surgery, the rats were furnished with food and water immediately freely.

Experimental groups

At the beginning of the study, forty male SD rats weighing 250-300g were randomized into four groups, each consisting of 10 rats (fig. 1).

Observation of adhesion formation

At the end of the procedures, all incisions were closed using full-thickness running 4/0 non-absorbable suture, and the rats were allowed to wake spontaneously. After 7 days, the rats were anesthetized with 10% chloral hydrate, then the peritoneal cavity was underwent via "U" incision without damaging the formed adhesions. In each group, 10 rats were decapitated and their abdominal cavities were opened by another two surgeons who were blind to the first surgery and not aware of the previous procedures. These surgeons evaluated the intra-peritoneal adhesions and graded the macroscopic adhesion scores based on the scale of Bigatti *et al* (Phillips and Dudley, 1984) (table 1, fig. 2). In addition, rates of mortality and wound infection were recorded.

STATISTICAL ANALYSIS

Data were presented as mean values ± standard deviation using SPSS17.0 (SPSS Inc, Chicago, Illinois, USA). Wilcoxon or Kruskal-Wallis test was used to compare the results of groups, followed by LSD test for determining the significance of difference between groups. Differences were considered significant for $P<0.05$ and standard test set α at 0.05.

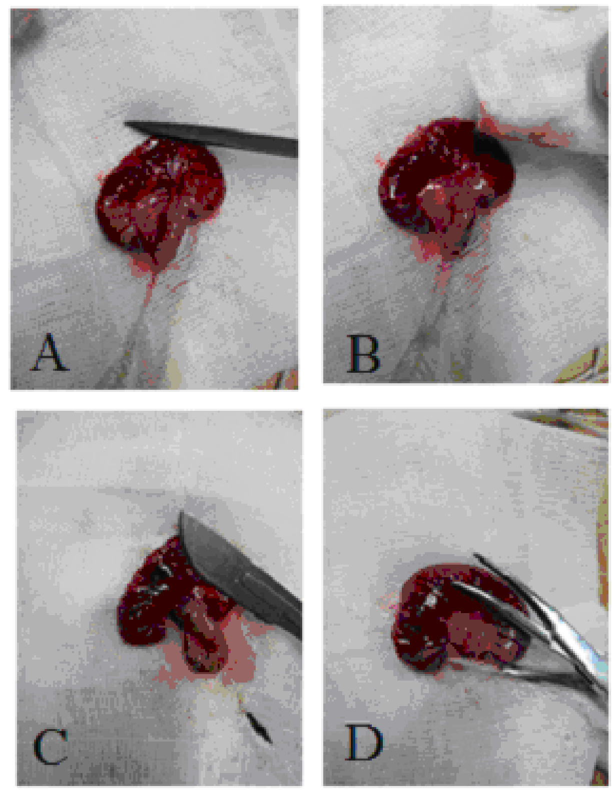

Fig. 1: Different Techniques inducing models of post-operative adhesion.

(A) A 2×3cm area of the right side of cecum was abraded with sterile rasp for 20 times until spot bleeding appeared (n=10); (B) A 2×3cm area of on the right side of cecum was abraded using sterile dry gauze at the ventral and dorsal surfaces until it lost its shine and hemorrhagic points became visible (n=10); (C) A 2×3cm area of on the right side of cecum was abraded using sterile blade until spot bleeding appeared (n=10); (D) A 2×3cm area of on the right side of cecum was abraded using vascular clamp until spot bleeding appeared (n=10).

RESULTS

Weight loss of forty rats was found after the initial surgery. In each group, rats slipped into poor condition of bad appetite and inactivity since the second day, but they recovered two days later, consumed more food and drink, and their activity, diet and water intake became normal on day 3. The mortality of rats was 7.5% (3/40, 2/10 rat of group C, 1/10 of group D). A number of reasons like intestinal stasis necrosis, bleeding, strangulated intestinal obstruction and peritonitis resulting from bowel perforation could account for the results via postmortem. All animals were euthanized for necropsy after ten days later, and the adhesion scores and average rank were displayed in table 2. In group A, ten postoperative adhesion rats induced by rasp presented adhesions, four developed score 3 adhesions, while six developed score 4 adhesions, most of them were well-formed, filamentous and broad which existed between peritoneum and adjacent liver, which proved that the peritoneal adhesion model is successfully established. Compared with group A, the area and quantity of adhesions was remarkably reduced in-group C. In the group B, the adhesions formed were nearly similar and dense as present in the group D.

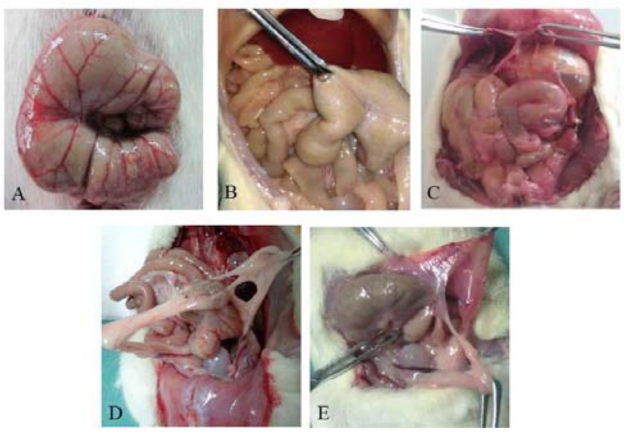

Fig. 2: Adhesion Score by Bigatti score system
(A. 0 score; B.1score; C.2 score; D.3 score; E.4 score)

Summary statistics for the different groups are shown in table 2. Kruskal-Wallis test was applied in data analysis, suggesting that 4 groups had significant difference (χ^2=14.92, P<0.0001). The median of the average rank were as follows: models of Group B and D reached score 1 of Bigatti Score System, Group A score 4, Group C score 3. Least-significant difference (LSD) test did not find any difference between group B and D (F=1.30, P=0.2619), but there was significant difference between A and B, C, D groups. (F=38.68, P<0.0001; F=6.25, P=0.0176; F=24.13, P<0.0001). In addition, statistical significance was also found between C and B, D groups. (F=11.32, P=0.0020; F=4.86, P=0. 0345) (table 2). There was remarkably difference in best performance in terms of both quantity and quality of adhesion formation among groups A, B, and C.

DISCUSSION

Post-operative adhesions after upper and lower abdominal surgery are responsible for significant morbidity and constitute an important public health problem. No technique, surgical approach, or commercial anti-adhesive agents has yet demonstrated its ability to reduce the risk of symptomatic intra-peritoneal adhesions. Gartner expressed their concerns in current adhesion models when they stated: ‘‘because there is no consistent, quantifiable, and reproducible model to establish a baseline, results have been difficult to evaluate’’ (Gartner *et al.,* 2008). Furthermore, it is also problematic to reach a clear-out conclusion from anti-adhesive proposals when no credible adhesion model is in place to verify these findings. A proper animal model that reflects different aspects of adhesions is crucial for evaluating various anti-adhesion strategies. The importance of this study is underscored that four different cecum models were compared with different technique, which is practical in clinic. In addition, statistical analysis was performed so as to determine the best model.

The present study compared four different models of rat cecum adhesions, finding the sterile rasp technique is the best for modeling. In the process of establishing the rats model of patholohical postoperative intraperitoneal adhesion, models by sterile dry gauze and vascular clamp harvested the score 1 according to Bigatti five score system, while model by rasp reached the score 5. Gauze and vascular clamps were inappropriate to build model, because they would cause false positive therapeutic effects and severely interfered the authenticity of experimental results. Models established by using blade reached score 3 but with high mortality that may produce the illusion of drug adverse reaction. Basically, they can be used for judging the effect of mediation, but more samples should be added in experiments in consideration of the loss. The best model was established by rasp, which reached the score 5 and whose adhesion surface was even and the mortality was 0, so rasp is the most appropriate molding tool to duplicate pathological postoperative intraperitoneal adhesion in rats’ cecum.

We analyzed its advantages as follows. First, degree of uniformity. The surface of sterile rasp is hard enough to abrade the uneven cecum surface to make the dorsal site as even as possible. The assorted file is of steel surface. Although the appendix surface is uneven, it can be held up by fingers on the back of the cecum to make an even side for abrasion. The surface of dry gauze is flexible, although it can self-adjusted when rubbing the uneven cecum, the abrasion force is not uniform. The gaps between vascular clamps will make the abrasion surface uneven, and the same problem exists when using sterile blade. Second, qualitative index. According to the above experiments, when sterile rasp is used to rub the serosa of cecum on the right side, the bleeding spots come out in the 2 X 3cm^2 wounded surface. This provided a comparatively objective index. The sterile rasp seems to be the comparatively appropriate tool to create an intra-abdominal adhesion model. One to three experimenter should be involved in the molding process and well managed. For example, one person is responsible for opening the abdomen, one for creating the model and the other for closing the abdomen.

Table 1: Adhesion Score by Bigatti *et al*.

Tenacity	Type	Extent (%)	Score
None	None	0	0
Adhesions essentially fell apart	Filmy, no vessels	<25	1
Adhesions lysed with traction	Dense, no vessels	25-50	2
Adhesions required blunt dissection	Dense, vascular, small vessels	50-75	3
Adhesions is difficult to dissect	Dense, vascular, large vessels	>75	4

Table 2: Adhesion scores and comparison of groups according to Bigatti adhesion scoring systems

Group	N	Bigatti Scores					Average rank
		0	1	2	3	4	
A	10	0	0	0	4	6	30.00
B	10	0	6	3	1	0	10.35*
C	8	0	0	2	6	0	21.63*
D	9	0	5	1	2	1	14.056*

*Indicates statistical significance as compared to group A ($P<0.05$).

There are several reports on creation of intra-abdominal adhesion models with different techniques; most of them compare one or two different techniques and the results vary. Their results on cecal abrasion were similar to what we found in our study, however, the sterile rasp technique appears superior, as the highest scores of adhesions was seen in this group.

A readily reproducible adhesion model will allow for better evaluation of the anti-adhesion agent because confounding results from the model will be minimized.

A weakness of this study was the limited number of animals used in this experiment. A larger study with a higher sample size and more scoring system need to be applied in near future.

In conclusion, the sterile rasp technique seems to be the most consistent and reproducible technique for an intra-abdominal adhesion model. Our statistical analysis demonstrate that there was enough power to detect a significant difference among four models. This method may be beneficial for experimental studies and the development of anti-adhesive agents in the near future.

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REFERENCES

Alkhamesi NA and Schlachta CM (2013). The role of aerosolized intraperitoneal heparin and hyal uronic acid in the prevention of postoperative abdominal adhesions. *Surg. Endosc.*, **27**(12): 4663-4669.

Alonso Jde M, Alves AL, Watanabe MJ, Rodrigues CA and Hussni CA (2014). Peritoneal response to abdominal surgery: The role of equine abdominal adhesions and current prophylactic strategies. *Vet. Med. Int.*, 279730.

Batukan C, Ozgun MT, Basbug M and Muderris II (2007). Sildenafil reduces postoperative adhesion formation in a rat uterine horn model. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **135**(2): 183-187.

Belluco C, Meggiolaro F, Pressato D, Pavesio A, Bigon E, Donà M, Forlin M, Nitti D and Lise M (2001). Prevention of postsurgical adhesions with an autocrosslinked hyaluronan derivative gel. *J. Surg. Res.*, **100**(2): 217-221.

Brüggmann D, Tchartchian G, Wallwiener M, Münstedt K, Tinneberg HR and Hackethal A (2010). Intra-abdominal adhesions: Definition, origin, significance in surgical practice, and treatment options. *Dtsch. Arztebl. Int.*, **107**(44): 769-775.

Burnett SH, Beus BJ, Avdiushko R, Qualls J, Kaplan AM and Cohen DA (2006). Development of peritoneal adhesions in macrophage depleted mice. *J. Surg. Res.*, **131**(2): 296-301.

Cashman JD, Kennah E, Shuto A, Winternitz C and Springate CM. (2011). Fucoidan film safely inhibits surgical adhesions in a rat model. *J. Surg. Res.*, **171**(2): 495-503.

Cassidy MR, Sherburne AC, Sheldon HK, Gainsbury ML, Heydrick S and Stucchi AF (2014). Histone deacetylase inhibitors decrease intra-abdominal adhesions with one intraoperative dose by reducing peritoneal fibrin deposition pathways. *Surgery*, **155**(2): 234-244.

Cheong YC, Laird SM, Li TC, Laird SM, Li TC, Shelton JB, Ledger WL and Cooke ID (2001). Peritoneal healing and adhesion formation/reformation. *Hum Reprod. Update*, **7**(6): 556-566.

Condon ET, Cahill RA, O'malley DB, Aherne NJ and Redmond HP (2007). Evaluation of postoperative peritoneal adhesion formation following perioperative nicotine administration. *J. Surg. Res.*, **140**(1): 135-138.

Dinarvand P, Hashemi SM, Seyedjafari E, Shabani I, Mohammadi-Sangcheshmeh A, Farhadian S and Soleimani M (2012). Function of poly (lactic-co-glycolic acid) nanofiber in reduction of adhesion bands. *J. Surg. Res.*, **172**(1): e1-e9.

Ergul E and Korukluoglu B (2007). Peritoneal adhesions: Facing the enemy. *Int. J. Surg.*, **6**(3): 253-260.

Gartner WB, Hagerman GF, Felemovicius I, Bonsack ME and Delaney JP (2008). Two experimental models for generating abdominal adhesions. *J. Surg. Res.*, **146**(2): 241-245.

Gorvy DA, Barrie Edwards G and Proudman CJ (2008). Intra-abdominal adhesions in horses: A retrospective evaluation of repeat laparotomy in 99 horses with acute gastrointestinal disease. *Vet. J.*, **175**(2): 194-201.

Hermans MH, Brown L and Darmoc M (2012). Adhesion prevention in an intraperitoneal wound model: Performance of two resorbable hemostats in a controlled study in rabbits. *J. Biomed. Mater. Res. B. Appl. Biomater.*, **100**(6): 1621-1626.

Hong JH, Choe JW, Kwon GY, Cho DY, Sohn DS, Kim SW, Woo YC, Lee CJ and Kang H (2011). The effects of barrier materials on reduction of pericardial adhesion formation in rabbits: A comparative study of a hyaluronan-based solution and a temperature sensitive poloxamer solution/gel material. *J. Surg. Res.*, **166**(2): 206-213.

Karaca T, Gözalan AU, Yoldaş Ö, Bilgin BÇ and Tezer A (2013). Effects of tamoxifen citrate on postoperative intra-abdominal adhesion in a rat model. *Int. J. Surg.*, **11**(1): 68-72.

Karacam V, Onen A, Sanli A, Gurel D, Kargi A, Karapolat S and Ozdemir N (2011). Prevention of pleural adhesions using a membrane containing polyethylene glycol in rats. *Int. J. Med. Sci.*, **8**(5): 380-386.

Kössi J, Salminen P, Rantala A and Laato M (2003). Population-based study of the surgical workload and economic impact of bowel obstruction caused by postoperative adhesions. *Br. J. Surg.*, **90**(11): 1441-1444.

Lai HS, Chen Y, Chang KJ and Chen WJ (2003). Effects of octreotide on epidermal growth factor receptor, tissue plasminogen activator and plasminogen activator inhibitor during intraperitoneal adhesion formation. *J. Gastroenterol.*, **38**(6): 555-560.

Lauder CI, Garcea G, Strickland A and Maddern GJ (2011). Use of a modified chitosan-dextran gel to prevent peritoneal adhesions in a rat model. *J. Surg. Res.*, **171**(2): 877-882.

Lauder CI, Strickland A and Maddern GJ (2012). Use of a modified chitosandextran gel to prevent peritoneal ad hesions in a porcine hemicolectomy model. *J. Surg. Res.*, **176**(2): 448-454.

Li S, Zhang YL, Sun JY, Hua YW and Wu PH (2013). Safe temperature range for intraoperative and early pos toperative continuous hyperthermic intraperitoneal perfusion in a swine model of experimental distal gastrectomy with Billroth II reconstruction. *J. Transl. Med.*, **11**: 181.

Lin HF, Wu CY, Wu MC, Chou TH, Lin GS, Yen ZS and Chen SC (2014). Hypothermia decreases postoperative intra-abdominal adhesion formation. *Am. J. Surg.*, **208**(3): 419-424.

Nunobe S, Hiki N, Fukunaga T, Tokunaga M, Ohyama S, Seto Y and Yamaguchi T (2008). Previous laparotomy is not a contraindication to laparoscopy-assisted gastrectomy for early gastric cancer. *World J. Surg.*, **32**(7): 1466-1472.

Ouaïssi M, Gaujoux S, Veyrie N, Denève E, Brigand C, Castel B, Duron JJ, Rault A, Slim K and Nocca D (2012). Post-operative adhesions after digestive surgery: their incidence and prevention: Review of the literature. *J. Visc. Surg.*, **149**(2): e104-e114.

Ozturk E, Yilmazlar A, Berhuni S and Yilmazlar T (2010). The effectiveness of local anesthetics in preventing pos toperative adhesions in rat models. *Tech. Coloproctol.*, **14**(4): 337-340.

Panahi F, Sadraie SH, Khoshmohabat H, Shahram E, Kaka G and Hosseinalipour M (2012). Macroscopic and pathological assessment of methylene blue and nor mal saline on postoperative adhesion formation in a rat cecum model. *Int. J. Surg.*, **10**(9): 537-541.

Park SN, Jang HJ, Choi YS, Cha JM, Son SY, Han SH, Kim JH, Lee WJ and Suh H (2007). Preparation and characterization of biodegradable anti-adhesive membrane for peritoneal wound healing. *J. Mater Sci. Mater. Med.*, **18**(3): 475-482.

Parker JE, Fubini SL and Todhunter RJ (1989). Retrospective evaluation of repeat celiotomy in 53 hors es with acute gastrointestinal disease. *Vet. Surg.*, **18**(6): 424-431.

Parker MC, Wilson MS, van Goor H, Moran BJ, Jeekel J, Duron JJ, Menzies D, Wexner SD and Ellis H (2007). Adhesions and colorectal surgery call for action. *Colorectal Dis.*, **9**(Suppl 2): 66-72.

Parsaei P, Karimi M, Asadi SY and Rafieian-Kopaei M (2013). Bioactive components and preventive effect of green tea (Camellia sinensis) extract on post-laparotomy intra-abdominal adhesion in rats. *Int. J. Surg.*, **11**(9): 811-815.

Pascual G, Sotomayor S, Rodríguez M, Bayon Y and Bellón JM (2013). Behaviour of a new composite mesh for the repair of full-thickness abdominal wall defects in a rabbit model. *PLoS One*, **8**(11): e80647.

Phillips RK and Dudley HA (1984). The effect of tetracycline lavage and trauma on visceral and parietal peritoneal ultrastructure and adhesion formation. *Br. J. Surg.*, **71**(7): 537-539.

Rajab T, Ahma d U and Kelly E (2010). Implications of late complications from adhesions for preoperative

informed consent. *J. R. Soc. Med.*, **103**(8): 317-321.
Ryan GB, Grobety J and Majno G (1971). Postoperative peritoneal adhesions. A study of the mechanisms. *Am. J. Pathol.*, **65**(1): 117-148.
Sahbaz A, Ersan F and Aydin S (2014). Effect of Nigella sativa oil on postoperative peritoneal adhesion formation. *J. Obstet. Gynaecol. Res.*, **40**(2): 532-537.
Schnuriger B, Barmparas G, Branco BC, Lustenberger T, Inaba K and Demetriades D (2011). Prevention of postoperative peritoneal adhesions: A review of the literature. *Am. J. Surg.*, **201**(1): 111-121.
ten Broek RP, Issa Y, van Santbrink EJ, Bouvy ND, Kruitwagen RF, Jeekel J, Bakkum EA, Rovers MM and van Goor H (2013). Burden of adhesions in abdominal and pelvic surgery: Systematic review and met-analysis. *BMJ.*, **347**: f5588.
Ward BC and Panitch A (2011). abdominal adhesions: Current and novel therapies. *J. Surg. Res.*, **165**(1): 91-111.
Yarema IV and Mogamedov MA (2003). Experimental study of the use of perftoran for preventing the formation of postoperative adhesions in peritonitis. *Bull. Exp. Biol. Med.*, **136**(6): 582-584.
Zhang QY, Ma S, Xi D, Zhang WT and Li AW (2011). Administration of a novel penicillamine-bound membrane: A preventive and therapeutic treatment for abdominal adhesions. *BMC. Surg.*, **11**: 5.
Zinther NB, Wara P and Friis-Andersen H (2010). Intraperitoneal onlay mesh: An experimental study of adhesion formation in a sheep model. *Hernia*, **14**(3): 283-289.