Ann Med Res 2021;28(8):1490-5

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.09.925

Original Article

Effect of pentoxifylline administration on an experimental rat model of intraperitoneal adhesion

Omer Lutfi Akgul[1], Erkan Dalbasi[2], Mesut Gul[3], Ulas Alabalik[4]

[1]Clinic of General Surgery, Genesis Hospital, Diyarbakir, Turkey
[2]Clinic of General Surgery, Memorial Hospital, Diyarbakir, Turkey
[3]Clinic of General Surgery, Medicalpark Hospital, Gaziantep, Turkey
[4]Department of Pathology, Faculty of Medicine, Dicle University, Diyarbakir, Turkey

Abstract

Aim: Intraabdominal adhesions are the pathological structures that form between the peritoneal surfaces throughout the healing of the peritoneal surface defects with scar formation. Adhesions are a significant problem because they affect the quality of life, lead to morbidity and mortality, and increase health expenses. We have investigated the effectiveness of pentoxifylline (PTX), which is a methyl xanthine derivative, on the development of experimentally generated intraperitoneal adhesions in rats.

Materials and Methods: In our study, 30 Wistar Albino rats, each weighing an average of 200-250 grams, were used. In this study, 3 groups of 10 rats were formed. As the adhesion model, cecotomy method was preferred and applied. Groups were classified as; the sham group, control group and PTX group. In the Sham group, merely laparotomy was applied, the control group was intraperitoneal 5 cc isotonic after the cecotomy, and the PTX group was intraperitoneal 100 mg / kg after the cecotomy. Rats were sacrificed on the 15th day. Macroscopic, microscopic adhesion and adhesion tissues were evaluated with regard to hydroxyproline levels.

Results: In the comparison of the groups from the macroscopic point of view, there was a noteworthy difference in adhesion between the sham group and the PTX group ($p = 0.027$) and between the control group and the PTX group ($p = 0.001$).

Conclusion: In this study, it was observed that PTX can reduce intraabdominal adhesion and increase the level of hydroxyproline after surgery.

Keywords: Adhesion; abdominal surgery; pentoxifylline

INTRODUCTION

Once the mesothelial layer of the peritoneal cavity is damaged as a result of the intra-abdominal adhesions injures due trauma, ischemia, inflammation and infection, fibrin is formed on the damaged surface as a result of bleeding and protein leakagetissue. Fibrin is a sticky substance that bonds the damaged tissues and which provides healing of the injured This procedure starts within three hours, on the fifth day, fibroblasts are concealed over the fibrin network, and adhesions are formed. These adhesions may be a thin connective tissue tape or a thick and fibrous band that is heavily bleeding. Typically, adhesions can occur between areas that cannot be covered by mesothelium, as well as between normal tissues in contact with the injured peritoneum surface (1,2). The most common reason of abdominal adhesions are former abdominal surgeries. It may cause severe complications such as chronic abdominal pain, bowel obstruction and infertility. Even though there have been many studies to prevent adhesions in the past, an effective method that has been used habitually has not been revealed. Intraabdominal adhesions remain to be a significant problem currently, as they affect the patient's quality of life, increase morbidity and mortality, and increase health expenses (3).

In our study we aimed to investigate, Pentoxyl as a methyl xanthine derivative, decreases blood viscosity by decreasing plasma fibrinogen concentration by increasing erythrocyte cell deformability, decreasing platelet adhesion and aggregation, decreasing leukocyte activation, and endothelial damage caused by leukocyte activation (decreasing the endothelial damage caused by leukocyte as a pentoxyl) and demonstrate its efficiency in the created intraabdominal adhesion model.

MATERIALS and METHODS

Study Design

This experimental study, carried out in this laboratory

Received: 08.09.2020 **Accepted:** 22.10.2020 **Available online:** 18.03.2021
Corresponding Author: Omer Lutfi Akgul, Clinic of General Surgery, Genesis Hospital, Diyarbakir, Turkey
E-mail: omerlutfi5@gmail.com

by obtaining the approval of the Ethical Committee of the Experimental Animals Local Ethics Committee of T. C. Diyarbakir Dicle University Prof.Dr. Dr. Sabahattin Payzin Health Sciences Research and Application Center Experimental Animals Local Ethics Committee dated 12.03.2013 and numbered 2012/59.

In the study, 30 Wistar Albino rats, each weighing an average of 200-250 grams, were used. The rats were kept at the room temperature and in average cages with a maximum of five groups in a 12-hour night and 12-hour day cycle. Ordinary laboratory food and water were given to the rats in the preoperative and postoperative period. After the adaptation process, three different groups were formed by randomizing, each being 10 rats: Group of control, Sham and Pentoxifylline (PTX). Pentoxifylline (Trental® CR) which used in the study was obtained from Sanofi Aventis. It is designed to be 100 mg / kg.

The operations were performed in sterile environments. Anesthesia of all rats was provided by intramuscular administration of 50 mg / kg ketamine hydrochloride (Ketalar®, Pfizer, Istanbul) and 5 mg / kg xylazine hydrochloride (Rompon®, Bayer, Şişli, Istanbul) under aseptic circumstances. After being anesthetized rats in a supine position by shaving the abdominal wall, and at that point povidone iodine (Isosol®, Central Lab. Ilac San. Turkey) and antisepsis were provided. A 3 cm midline incision was prepared after anesthesia to apply the adhesion model. After determining that there is no adhesion in the abdomen, the cecum was exposed. Approximately 1 cm of cecotomy was implemented on the antimesenteric side of the cecum taken out of the abdomen with a number 11 scalpel. The cecotomy was restored constantly on a single coat with 4/0 vicryl and placed in the first place in the abdomen. Consequently, the abdominal wall of the rats was sutured with 3/0 vicryl and the skin was sutured one by one with 3/0 silk sutures.

To form the sham group, the abdominal walls of 10 rats, which were found to have no adhesion following the midline incision, were uninterruptedly sutured with 3/0 vicryl, and the skin was individually sutured with 3/0 silk sutures. Subsequently applying the adhesion model to 10 rats in the control group, 5 cc isotonic was given to them intraperitoneally into the abdomen. Later applying adhesion model to 10 rats in the PTX group, 100 mg / kg PTX was given intraperitoneally into the abdomen.

Later the rats were managed intravenously with Ketamine and Xylazine at the ordinary anesthesia dose on the fifteenth day; sacrification was obtained by taking intracardiac blood. Consequently, the abdomen was retracted downwards with a U incision and thus maximum vision was accomplished.

Adhesions at the macroscopic level were evaluated quantitatively considering the Mazuji classification. The assessment was carried out by two distinct persons, in accordance with the classification formerly described to them, and the way of double-blind (Table 1) (4).

Table 1. Mazuji's macroscopic adhesion classification

Grade 0: No adhesion
Grade 1: Very fine and fragmented irregüler adhesion
Grade 2: Medium density and fragmented adhesion that can be separated easily
Grade 3: Intense, not easily separable regular adhesion
Grade 4: Very intense, not easily separable, homogeneous adhesion

Following the macroscopic evaluation, whereas the affected organs were excised together with the tape in the adhesions emerging adhesions, the cecum was excised for pathological sample including all layers except the anterior wall and parietal peritoneum skin. The pathological samplings were then fixed in containers which hold 10% buffered formaline. Tracks followed by classical laboratory method were embedded in paraffin blocks and 5 micrometer sections were taken on the slide. It was stained with hematoxylin-Eosin dye and examined by light microscopy. The pathologist who made the inspection wasn't aware of from which group the fragments were taken. After histopathological assessment, the fragments were subjected to microscopic grading defined by Zühlke (Table 2) (5,6).

Table 2. Zühlke's microscopic adhesion classification

Grade 1: Weak connective tissue, rich cell, new and old fibrin, thin reticulin fibriles
Grade 2: Connective tissue which has cells and capillaries. Few collagen fibers
Grade 3: Thicker connective tissue. Few cells and elastic and smooth muscle fibers, more vessels
Grade 4: Old firm granulation tissue, cell-poor, serosal layers that are hardly distinguishable

From the tissues taken for histopathological sampling, 1 gram was weighed and hydrolyzed at 121 degrees for 5 hours in 1 ml of acidic buffer. Then, the working material was obtained by centrifuging at 5000 rpm for 20 minutes. Tissue hydroxyproline level was calculated as mg / L / gr / tissue by assessing the absorbance of the formed material at 121 degrees at 560 nm (colorimetric). (Used Kit Lot No: 41100, Kit: Hydroxyproline Extra, Colorimetric Determination, Brand: Far / Italy).

Statistical Analysis

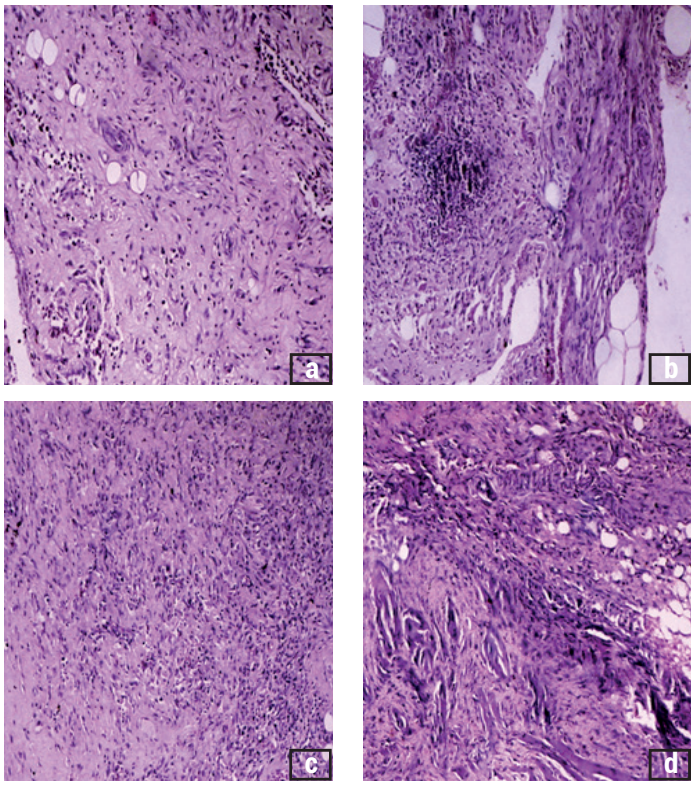
While assessing the findings acquired in the study, SPSS (Statistical Package for Social Sciences) for Windows 17.0 program was used for statistical analysis. Results were estimated at 95% confidence interval and $p < 0.05$. Descriptive statistical methods (frequency count, mean, standard deviation of the mean) were used to calculate the study data. Chi-Square and Fisher's exact test were

used to compare classified data between groups. Non-parametric methods considering the small size of the sample included in the study; Kruskal Wallis Analysis, Mann Whitney U Test was used. In addition, Bonferroni and Tamhane tests, which are among the Anova and Post Hoc tests, were used. Anova test was applied to macroscopic and microscopic results. One-way Anova test was used to evaluate variance homogeneity in groups. Bonferroni and Tamhane tests, which are among the Post Hoc tests, were used to find out which group caused the difference.

RESULTS

There was no loss of subjects throughout the experimental study. Congenital adhesion was not detected in the first laparotomy. Incisional hernia and wound dehiscence were not noticed in rats in which experimental study was performed.

In histopathological assessment, the views of grade 1, grade 2, grade 3, grade 4 in the materials of the subjects graded according to the Zühlke classification are shown in Figure 1, respectively. The data obtained by determining the hydroxyproline level is shown in Table 3.



a. grade 1 microscopic image; b. grade 2 microscopic image; c. grade 3 microscopic image; d. grade 4 microscopic image

Figure 1. Histopathological specimen images

Kruskal Wallis test of macroscopic values (X2 = 13,779; P = 0.001); in addition, the results of the Post Hoc Bonferroni multiple comparison test applied after the ANOVA (F = 9.26; P = 0.001) test are given in Table 4 and 5.

When macroscopic results are assessed statistically; There was no substantial difference in adhesion between the sham group and the control group (p = 0.511). There was a substantial difference in adhesion between the control group and the PTX group (p = 0.001). In PTX group, adhesion was found to be considerably less macroscopically than the control group. There was a significant difference in adhesion between the Sham group and the PTX group (p=0.027). Comparison of macroscopic results between groups are specified in Figure 2.

Table 3. Tissue hydroxyproline values by groups (mg/L/g/doku)

Subject	Sham	Control	Pentoxifylline
1	82.68	47.25	117
2	47.25	56.25	92.25
3	59.06	56.25	63
4	70.87	38.25	60.75
5	59.06	58.50	56.25
6	47.25	51.75	78.75
7	82.68	40.50	112.50
8	70.87	65.25	78.75
9	70.87	45	60.75
10	47.25	24.75	101.25

Table 4. ANOVA test results

	Subject	Average	Standard Deviation	95% Security	Interval
Macroscopic					
Sham	10	1.50	0.52	1.12	1.88
View					
Control	10	2	1.15	1.17	2.83
PTX	10	0.50	0.52	0.12	0.88
Microscopic					
Sham	10	1.10	0.31	0.87	1.33
View					
Control	10	3.20	0.78	2.64	3.76
PTX	10	1.60	0.51	1.23	1.97

PTX: Pentoxifylline

Table 5. Post Hoc Bonferronia test results of macroscopic values

Macroscopi	P(<0.05)	Average Difference
Sham - Control	0.511	-0.50
Control - PTX	0.001	1.50
Sham - PTX	0.027	1

PTX: Pentoxifylline

Kruskal-Wallis test of microscopic values was applied (X2=21.052; P=0.001), In addition, the results of the Post Hoc TAMHANE multiple comparison test performed after the ANOVA test (F= 36.50; P= 0.001) are given in Table 6.

Once microscopic results are assessed statistically; there was a noteworthy difference in adhesion between the sham group and the control group (p=0.0001).

There was a noteworthy difference in adhesion between the control group and the PTX group (p=0.0001). Adhesion was found to be suggestively higher than the other two groups in the control group. Adhesion in the PTX group compared to the control group was found to be suggestively less microscopically. There was no important difference in adhesion between the Sham group and the PTX group (p=0.058). Comparisons of microscopic results are given in Figure 3.

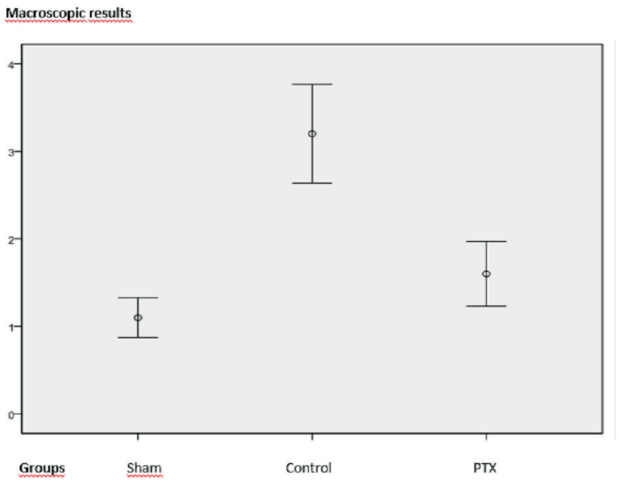


Figure 2. Comparison of macroscopic results

Table 6. Post Hoc Tamhane test results of microscopic values		
Microscopi	P(<0.05)	Average Difference
Sham - Control	0.0001	-2.10
Control - PTX	0.0001	1.60
Sham - PTX	0.058	-0.50
PTX: Pentoxifylline		

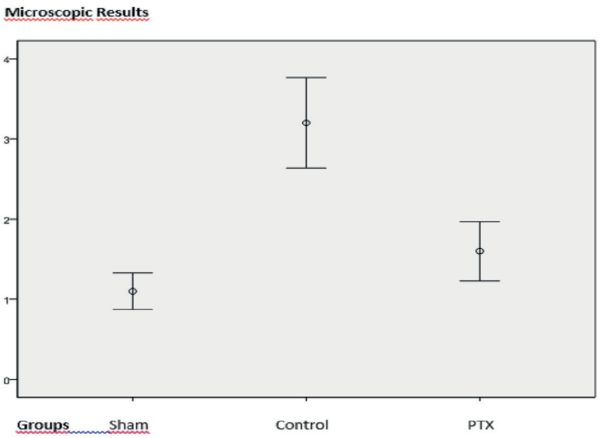


Figure 3. Comparison of microscopic results

After hydroxyproline levels are assessed statistically; there was a important difference between the sham group and the control group in terms of hydroxyproline amount (p=0.046). There was a momentous difference between the control group and PTX group in terms of hydroxyproline amount (p=0.03). The amount of hydroxyproline was highest in the PTX group. No important difference was found between Sham group and PTX group in terms of hydroxyproline amount (p=0.128). A comparison of hydroxyproline levels are given in Figure 4. ANOVA test was used to compare tissue hydroxyproline levels. The results are shown in Table 7. Kruskal-Wallis test of hydroxyproline levels (X2=13,46; P=0,001). In addition, the results of the Post Hoc Tamhane's multiple comparison test applied after the ANOVA test (F= 10.186; P= 0.001); are given in Table 8.

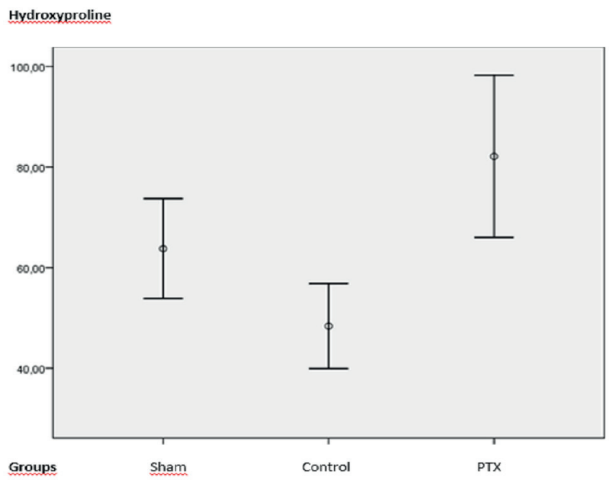


Figure 4. Comparison of hydroxyproline levels

Table 7. ANOVA test results of hydroxyproline levels							
	N	**Average**	**Standard Deviation**	**Min**	**Max**	**95% Security**	**Interval**
Sham	10	63.78	13.86	47.25	82.68	53.86	73.70
Control	10	48.37	11.82	24.75	65.25	39.91	56.83
PTX	10	82.12	22.55	56.25	117.00	65.98	98.26
Total	30	64.76	21.39	24.75	117.00	56.77	72.75
PTX: Pentoxifylline							

Table 8. Post HocTAMHANE test results of hydroxyproline levels		
Hydroxyproline	P(<0.05)	Average Difference
Sham - Control	0.046	15.40
Control - PTX	0.030	-33.75
Sham - PTX	0.128	-18.34
PTX: Pentoxifylline		

DISCUSSION

In the current study, PTX was found to be effective in preventing adhesion, both microscopically and macroscopically. In addition, the hydroxyproline level increased the most in the PTX group.

To decrease intra-abdominal adhesions, effort should be on preventing tissue damage, using suitable sewing materials and surgical techniques, keeping the operation time as short as possible and reducing intra-peritoneal exudates. Adhesions basis augmented morbidity, mortality

and cost due to severe complications such as intestinal obstruction, infertility, chronic abdominal and pelvic pain, and complicating subsequent surgical interventions (7).

It comprises diverse pathological procedures such as the development of postoperative intra-abdominal adhesion, posttraumatic immune activation, inflammatory response and fibrinolysis imbalance (8). For that reason, ideal adhesion inhibitors; it should not damage peritoneal mesothelial cells, that is, it should be complete intact. It should accelerate peritoneal wound healing and be fully absorbed. If it is not completely absorbed, it may cause more adhesion by creating granulomas. Absorption time should be between two and five days in agreement with the pathophysiology of adhesion formation. It must have viscosity to provide separation between surfaces (9).

Most of the studies to inhibit postoperative intraabdominal adhesion formation have been done with trauma models formed on peritoneal and serosal surfaces. To produce peritoneal adhesions, injured uterus horn model, peritoneal damage model, ileal transection model, colon anastomosis model, small intestine anastomosis model, bacterial peritonitis model, and clamping model, sponge disc implantation into the peritoneum, cecal abrasion models were used (10,11).

The Cecotomy model we use in our study is the recent adhesion model. Dalkılıç et al. (12), by using this model, they examined the effect of intraperitoneal taurolidine use on adhesions after surgery in rats. In addition to this, they examined the effect of modified chitosan-dextran gel in preventing peritoneal adhesions in rats after the use of the cecotomy adhesion model Lauder and et al. (13) and obtained positive results to prevent adhesion.

Pentoxifylline is a xanthine-derived phosphodiesterase inhibitor drug while Pentoxifylline is a effective peripheral vasodilator. Its main therapeutic efficacy is due to its hemorheologic effects, which aggregates blood flow and oxygenation of tissues (14). In studies realized with PTX, high doses such as 100 mg / kg and 200 mg / kg have been used and its advantageous effect has been shown to increase dose-dependent (15). Nevertheless, toxic effects have been reported in treatments of 200 mg / kg and above (16). In our study, PTX was used at a dose of 100 mg / kg.

In ordinary human dermal fibroblast culture, PTX has been revealed to impede IL-1 induced proliferation whereas at the same time stimulating collagen synthesis, glucose aminoglycan and fibronectin production, while stimulating collagenase activity (17).

A small number of studies of PTX were found in the inhibition of intraabdominal adhesions when the literature was reviewed. In the Kaleli et al. rat uterine horn model they found that pentoxifylline reduced intraabdominal adhesions (18). They informed that it decreased intraperitoneal adhesion formation in anastomosis region after intestinal resection in lai et al rats (19). Chalkiadakis et al. showed that it reduced adhesion in a peritonitis-induced rat model (20). Steinleitner et al. showed that pentoxifylline did not decrease adhesion development in rabbit uterine horn model (21). Tarhan et al. showed that intraperitoneal and intravenous pentoxifylline decreased adhesion development due to activation of the peritoneal fibrinolysis system in rats given cecal abrasion (22). Mendes et al. showed that cilostazol and pentoxifylline decrease the adhesion development by decreasing angiogenesis, inflammation and fibrosis in the intraabdominal adhesion model produced by sponge disc implantation into the peritoneum (23). Boztosun et al. showed the effect of methylene blue, pentoxifylline and enoxaparin on intraperitoneal adhesion in the rat uterine horn model. In this study, the result of pentoxifylline on adhesion formation was not determined and methylene blue and enoxaparin decreased adhesion formation. They found that methylene blue decreased adhesion markers by performing on angiogenesis (24). Yang YL et al. In their experimental study on rats, they applied 100 mg / kg PTX intraperitoneal quotidian, starting two days before surgery, and revealed that they reduced intraabdominal adhesion. In the same study, they found that PTX increased the protein levels in the adhesion area and reduced the number of ki67 + / CD31 + cells. Lastly, in the PTX-treated group, they observed a decrease in the expression of F4 / 80 +, FSP-1 + and a-SMA + cells at the adhesion site (25). In our study, we found that PTX reduced macroscopic and microscopic intraabdominal adhesions in accordance with the literature.

Numerous studies have used tissue hydroxyproline level as an indicator of collagen quantity. Hydroxyproline is mainly found in collagen. It shows 13% of the amino acid content of the molecule. It contains glycine, proline, hydroxyproline, hydroxyzine in its collagen content. Hydroxyproline and hydroxyzine are collagen-specific amino acids (26).

High tissue hydroxyproline level indicates high collagen synthesis. The tissue level of hydroxyproline, one of the main ingredients of collagen, is a decent indicator of the wound healing process (27). In our study, hydroxyproline level was higher in PTX group.

CONCLUSION

As a result of the experimental study we conducted to prevent intra-abdominal adhesions, which is still a serious problem today, we can say that; PTX can reduce intraabdominal adhesion and increase tissue hydroxyproline level after surgery.

Competing Interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical Approval: Research project on Effect of Pentoxifylline Administration on an Experimental Rat Model of Intraperitoneal Adhesion with protocol number 2012/59 was approved by the ethics committee.

REFERENCES

1. Herrick EH, Mutsaers SE, Ozua P, et al. Human peritoneal adhesions are highly cellular, innervated and vascularized. J Pathol 2000;192:67-72.

2. Hellebrekers BW, Trimbos-Kemper TC, Trimbos JB, et al. Use of fibrinolytic agents in the prevention of postoperative adhesion formation. Fertil Steril 2000;74:203-12.
3. Iwasaki K, Ahmadi AR. Qi L, Chen M, et al. Pharmacological Mobilization and Recruitment of Stem Cells in Rats Stops Abdominal Adhesions After Laparotomy. Scientific reports, 2019;1:1-8.
4. Mazuji MK, Kalmbaheti K, Powar B. Prevention of adhesions with polyviylpyrolidone Preliminary Report Arch Surg 1964;89:1011-5.
5. Reed KL, Fruin AB, Bishop-Bartolomei KK, et al. Neurokinin-1 receptor and substance P messenger RNA levels increase during intraabdominal adhesion formation. J Surg Res 2002;108:165-72.
6. Ersoy G, Utkan Z, Gokgoz S, et al. The effects of verapamil and pentoxifylline in the prevention of Intra-abdominal adhesions. Turkish Journal of Surgery 1992;8:229-34.
7. Risberg B. Adhesions: Preventive strategies. Eur J Surg 1997;577:32-9.
8. Hong G, Vilz TO, Kalff JC, et al. Peritoneal adhesion formation. Chirurg 2015;86:175-80.
9. Aysan E, Demir M, Kinaci E, et al. Complications of intestinal milking: experimental model. ANZ J Surg 2005;75:322-5.
10. Guzelsagaltici N, Girgin S, Gedik E, et al. Intraperitoneal octenidindihydro-chloride – phenoxyethanol solution to prevent peritoneal adhesion formation in a rat peritonitis model. Acta Obstet Gynecol Scand. 2007;86:395-400.
11. Yan S, Yue Y, Zeng L, et.al. Ligustrazine nanoparticles nano spray's activation on Nrf2/ARE pathway in oxidative stress injury in rats with postoperative abdominal adhesion. Ann Transl Med 2019;7:379.
12. Dalkilic G, Gul AE, Gulmen M. Sıçanlarda intraperitoneal taurolidin kullanımının cerrahi sonrası yapışıklıklara etkisi. Kartal Eğitim ve Araştırma Hastanesi Tıp Dergisi. 2007;18:113-8.
13. Lauder CIW, Garcea G, Strickland A, et al. Use of a modified Chitosan-Dextran gel to prevent peritoneal adhesions in a rat model. J Surg Res 2011;171:877-82.
14. Ward A, Clissold S. Pentoxifylline: A review of its pharmacodynamics and pharmacokinetic properties, and its therapeutic efficacy. Drugs 1987;34:50-97.
15. Santucci L, Fiorucci S, Giansanti M, et al. Pentoxifylline prevents indomethacin induced acute gastric mucosal damage in rats; role of tumour necrosis factor alpha. Gut 1994;35:909-15.
16. Yonetci N, Kosekli MA, Ozutemiz O, et al. Effects of pentoxifylline on alcohol-induced gastric injury and acid secretion in rats. Dig Dis Sci 2003;48:1392-6.
17. Lefaix JL, Delanian S, Vozenin MC, et al. Striking regression of subcutaneous fibrosis induced by high doses of gamma rays using a combination of pentoxifylinne and α-tocopherol: an experimental study. Int J Radiation Oncology Biol Phys 1999;43:839-47.
18. Kaleli B, Ozden A, Aybek Z, et al. The effect of L-Arginine and Pentoxifylline on postoperative adhesion formation. Acta Obstet Gynecol Scand 1998;77:377-80.
19. Lai HS, Chu SY, Chen Y, et al. Effect of pentoxifylline on intraperitoneal adhesions after intestinal resection in rats. J Formos Med Assoc 1994;93:911-5.
20. Chalkiadakis GE, Kostakis A, Karayannacos PE, et al. Pentoxifylline in the treatment of experimental peritonitis in rats. Arch Surg 1985;120:1141-4.
21. Steinleitner A, Lambert H, Kazensky C, et al. Pentoxifylline, a methylxanthine derivative, prevents postsurgical adhesion reformation in rabbits. Obstet Gynecol 1990;75:926-8.
22. Tarhan OR, Barut I, Sutcu R, et al. Pentoxifylline, a methyl xanthine derivative, reduces peritoneal adhesions and increases peritoneal fibrinolysis in rats. Tohoku J Exp Med 2006;209:249-55.
23. Mendes JB, Campos PP, Rocha MA, et al. Cilostazol and pentoxifylline decrease angiogenesis, inflammation, and fibrosis in sponge-induced intraperitoneal adhesion in mice. J Life Sci 2009;84:537-43.
24. Boztosun A, Picnak A, Kosar MI, et al. Effects of methylene blue, pentoxyphylline and enoxaparin on postoperative adhesion formation and markers of angiogenesis in a rat uterine horn model. Clin Exp Obstet Gynecol 2012;39:89-95.
25. Yang YL, Lee MG, Lee CC, et.al. Pentoxifylline decreases post-operative intra-abdominal adhesion formation in an animal model. Peer J 2018;6:e5434.
26. Garnero P, Delmas PD. Biochemical markers of bone turnover: Applications for osteoporosis. Endocrinol Metab Clin North Am 1998;27:303-23.
27. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses. III. Collagen metabolism in the colon after left colon resection. Am J Surg 1980;139:398-405.