Original Research

Intraperitoneal Application of Honey for Preventing Postoperative Intra-Abdominal Adhesions in Rabbits

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Abstract

The present study was conducted to evaluate the anti-adhesive properties of honey in preventing postoperative intraperitoneal adhesions in rabbits. Twelve healthy rabbits of either sex were randomly divided into two groups (Group C and T), comprising of 6 rabbits in each. Laparotomy was performed under anaesthesia in all the rabbits and scarification of caecum and ileum was performed. Honey was applied on the scarified surface in group T and no treatment was provided in group C. Evaluation for intraperitoneal adhesions carried out 10th and 21st post-operative day by reopening the abdomen revealed adhesion formation in four rabbits in group C compares to two in group T.

Key words: Honey, Intraperitoneal Adhesions, Laparotomy, Rabbit

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Introduction

Adhesions are the bands of tissue that connect organs together (Kamel, 2010). Postoperative intra-abdominal adhesion formation has been considered an inevitable consequence of laparotomy and the incidence in abdominal surgery is ranging between 67 and 93% (Cassidy *et al*., 2014 and Yildiz *et al*., 2015). Adhesions can develop when inflammatory responses disturb the equilibrium between fibrin formation and fibrinolysis (Emre *et al*., 2009) and it represents an important clinical challenge in gastro-intestinal surgery (Mahdy *et al*., 2008). Intra-abdominal adhesion formation and reformation after surgery are still unavoidable events inspite of modern surgical techniques (Ellis, 2004) and there is no satisfactory treatment or prophylaxis to deal with preventing adhesions (Roman *et al*., 2005).

Honey is a heterogeneous substance that inhibits the growth of both gram-positive and gram-negative bacteria, has anti-inflammatory effects, promotes healing processes following peritoneal damage and preventing postoperative peritoneal adhesions (Aysan *et al.*, 2002, Emre, 2009; Carnwath *et al.*, 2014 and Cooke *et al.*, 2015) in rats. In the present study, effect of honey was evaluated in preventing post-operative peritoneal adhesions in rabbits.

Materials and Methods

The study was conducted in the Department of Surgery and Radiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram. Twelve numbers of healthy rabbits of either sex was randomly divided into two groups (Group-C and Group-T), comprising of six (6) animals in each. Rabbits were clinically examined prior to surgery. Ventral abdomen prepared for aseptic surgery and was anaesthetized glycopyrrolate @ 0.1mg/kg IM, xylazine @4mg/kg IM and ketamine @ 20mg/ kg IM, respectively.

Rabbits were placed in dorsal recumbency and caudal midline laparotomy incision was made and ileum and caecum were exposed and the apex was abraded by scrapping until haemorrhagic points developed on the surface without perforation (Giusto *et al.*, 2017). In group T, honey was applied topically on the abraded surface and no treatment was provided in group C and laparotomy incision was sutured following standard surgical procedures. On 0, 7th, 14th and 21st post-operative days clinico-physiological (Heart rate, respiratory rate and rectal temperature) examination, physical and biochemical examination of peritoneal fluid and haematological examination of whole blood for packed cell volume, total leucocyte count and differential leukocytes count was carried out. On 10th and 21st post-operative days surgically reopening of abdomen were carried out in three rabbits of each group for the macroscopic assessment of adhesions and adhesion were recorded as per the method described by Blauer and Collins, 1988 (Table 1) and histopathological study of injured ileum and caecum walls was carried out to study the adhesion as per the method described by Aysan *et al.*, 2002. The recorded data were statistically analyzed using completely randomized design with the group comparison and two samples T-test for between the group comparison, following the methods described by Snedecor and Cochran (1994).

Table 1: Grading of adhesions (Blauer and Collins, 1988)

Grade	Description of adhesions
0	No adhesion
1	Thin adhesive bands easily removable
2	Thick band limited to one area
3	Extensive and thick adhesive bands
4	Extensive and thick adhesive bands and adhesions between viscera and/or abdominal wall.

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Results and Discussion

The clinico-physiological parameters which included heart rate, respiratory rate and rectal temperature recorded at different days in both the groups during study period were insignificant ($p>0.05$) with mild variations but were within the normal physiological limit (Table 2). Similar mild variations of heart rate, respiratory rate and rectal temperature were also observed by Loeser (2000), Chakrabarti (2006) and Kohn *et al.* (2007) following post-operative stress and trauma in animals.

Table 2: Mean ± SE values of heart rate (beats/min), respiratory rate (breaths/min) and rectal temperature (^{0}C) at different time intervals in groups C and T

Observations	Group	0 day	7th day	14th day	21st day
Heart rate (beats/min)	C	182.16 ± 1.42	182.5 ± 1.63	183.00 ± 1.63	181.33 ± 0.88
	H	180.16 ± 1.57	179.00 ± 1.06	181.33 ± 1.42	180.16 ± 1.05
Respiratory rate (breaths/min)	C	182.16 ± 1.42	182.5 ± 1.63	183.00 ± 1.63	181.33 ± 0.88
	H	180.16 ± 1.57	179.00 ± 1.06	181.33 ± 1.42	180.16 ± 1.05
Rectal temperature (^{0}C)	C	38.37 ± 0.66	37.94 ± 0.43	38.12 ± 0.34	38.27 ± 0.38
	H	38.61 ± 0.43	38.37 ± 0.14	38.26 ± 0.39	38.46 ± 0.26

Packed cell volume, total leukocyte count and differential leukocyte count did not show significant ($p>0.05$) changes during the study period though mild fluctuation were observed in both the groups but were well within the normal physiological range (Table 3). Similar insignificant changes in haematological parameters were also reported by Armitage-Chan and Baines (2012), Washington and Van Hoosier (2012) and Moore *et al*. (2015) during post-surgery and it might be due to inflammation, haemorrhage and chronic stress.

Table 3: Mean ± SE values of packed cell volume (%), total leukocyte count (M/mm^3), lymphocytes (%), monocytes (%) and granulocytes (%) at different time intervals in groups C and T

Observations	Group	0day	7thday	14thday	21stday
Packed cell volume (%)	C	43.66 ± 2.18	43.98 ± 1.96	48.45 ± 0.90	47.30 ± 1.04
	H	44.00 ± 2.69	44.33 ± 1.62	46.93 ± 1.37	46.15 ± 1.78
Total leukocyte count (M/mm^3)	C	6.17 ± 0.29	6.16 ± 0.48	6.72 ± 0.41	6.29 ± 0.39
	H	6.33 ± 0.24	6.53 ± 0.31	6.19 ± 0.36	5.99 ± 0.34
Lymphocytes (%)	C	37.96 ± 1.22	37.41 ± 1.62	34.21 ± 1.42	38.97 ± 0.72
	H	37.73 ± 1.01	38.56 ± 1.41	35.63 ± 1.51	38.23 ± 0.91
Monocytes (%)	C	6.96 ± 0.23	7.08 ± 0.55	7.35 ± 0.55	6.91 ± 0.37
	H	7.10 ± 0.25	7.15 ± 0.38	7.30 ± 0.29	7.05 ± 0.33
Granulocytes (%)	C	55.06 ± 1.14	55.50 ± 1.86	58.43 ± 1.03	54.78 ± 0.80
	H	55.16 ± 0.83	54.16 ± 1.74	57.06 ± 1.49	54.71 ± 0.97

Evaluation of peritoneal fluid showed slightly turbid and blood tinged colour with mild coagulation without odour in group C, while in group T resulted straw coloured and blood tinged peritoneal fluid without turbidity, coagulation and odour was observed. Similar peritoneal fluid observation was also reported by Reed *et al.* (2004) and Smith (2015). Turbidity and coagulation in group C might be due to increased in

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cells, proteins and fibrin meshwork in peritoneal fluid and serosanguinous fluid was an indication of either free erythrocytes or haemoglobin. The peritoneal fluid protein recorded in group C on 7th day (2.98 ± 0.21 g/dl) and 14th day (3.00 ± 0.11 g/dl) was slightly higher than the total protein concentration in normal peritoneal fluid (2.5 g/dl), although, in group C and T on other days, the total protein values were within normal range (Table 4). Increased total protein in peritoneal fluid was also reported by Binda *et al.* (2003) post operatively in animals due to peritoneal injury, surgery and irritation which initiates an inflammatory reaction.

Table 4: Mean ± SE values of total protein (g/dl) at different time intervals in groups C and T

Group	0day	7thday	14thday	21stday
C	2.36 ± 0.21	2.98 ± 0.21	3.00 ± 0.11	2.41 ± 0.26
H	2.36 ± 0.21	2.26 ± 0.25	2.36 ± 0.33	2.56 ± 0.23

On 10th post-operative day, three animals in group C revealed adhesions; out of which two was recorded as grade 3 and other with grade (Fig. 1). The animals of group T was recorded with grade 1 adhesion in one rabbit only. Similar observations of adhesions formation were also reported by Aysan *et al*. (2002) on 10th post-surgery day when honey was used in caecum and terminal ileum in rats.

Fig. 1: Thick adhesive bands between caecum and peritoneum in group C on 10th day

On 21st post-operative day, three rabbits of group C were observed with adhesions out of which two rabbits, with grade 1 adhesion and with grade 4 adhesions in another rabbit (Fig. 2). The animals of group T was recorded with grade 2 adhesions in one rabbit (Fig. 3). Similar observations were also reported by Avital *et al.* (2005) on 21st day post-operation with polylactic acid film (SurgiWrapTM) placed between the cecal and abdominal wall defects in rat.

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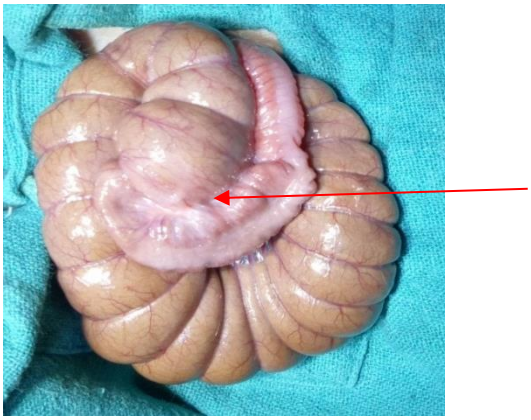

Fig. 2: Thick adhesive bands of adhesions between viscera in group C on 21st day

Fig. 3: Thick adhesive band limited to one area between caecum and peritoneum in group T on 21st day

Overall incidence of adhesions in group C was 4/6 rabbits (66.67%) and in group T was 2/6 rabbits (33.33%) (Table 5). Aysan *et al.* (2002) and Emre *et al.* (2009) were also observed lower grade and decreased incidence of adhesions with honey in rats. The decreased adhesion recorded in honey group might be due to the honey that inhibited the growth of both gram-positive and gram-negative bacteria, anti-inflammatory effects, and promotes healing processes following peritoneal damage (Carnwath *et al.*, 2014).

Table 5: Overall incidences of adhesions

	Day		Total (N=6)
Group	**10th day (N=3)**	**21st day (N=3)**	
Control	2	2	4
Honey	1	1	2

In group C, histopathological slides of the adhesive area, on 10th day showed remarkably extensive and multi-layered fibrosis with disorganized proliferation of fibroblasts and connective tissue fibres which were attached with peritoneum muscle tissue (H&E stain) (Fig. 4) and on 21st day were observed with adhesions between caecum and ileum, which showed extensive fibrosis with disorganized proliferation of fibroblasts and connective tissue fibres. There was remarkably extensive and multi-layered fibrosis with disorganized

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proliferation of fibroblasts and connective tissue fibres which were attached with abdominal muscle tissue with formation of vasculature which was congested with blood (H&E stain) (Fig. 5). On 21st post-operative day in group T, the histopathology showed deep blue staining collagen fibres with red stained RBC's and extensive fibrosis with disorganized proliferation of collagen fibres (Masson's Trichrome stain) (Fig. 6).

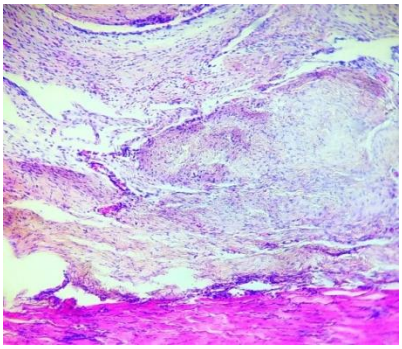

Fig. 4: Adhesions between intestine and peritoneal muscle on 10th day in group C (H & E stain X40).

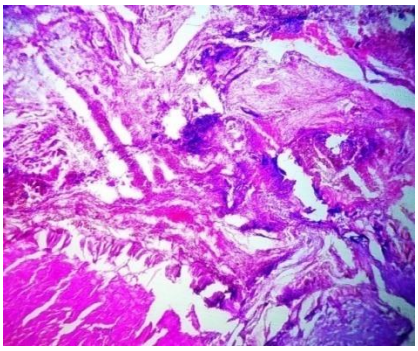

Fig. 5: Adhesions between intestine and peritoneal muscles on 21st day in group C (H & E stain X40)

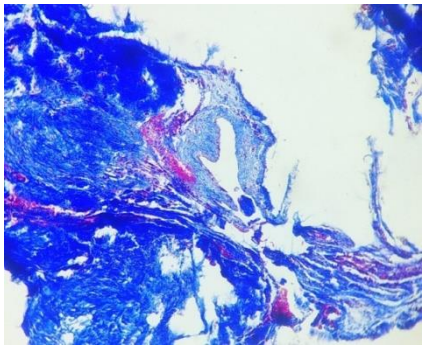

Fig. 6: Adhesions between intestine and peritoneal muscles on 21stday in group T (Masson's Trichrome stain, X40)

Non-specific inflammatory changes in the damaged caecal and terminal ileal walls in all specimens were recorded in the present study. Similar non-specific inflammatory changes were also observed by Aysan *et al.* (2002), Emre *et al.* (2009), Giusto *et al*. (2017) and Hatipoglu *et al*. (2017). The extensive fibroblast with disorganized proliferation of collagen fibres recorded in the present study might be due to lower fibrinolytic activity in rabbit's tissues (Dörr, 1990).

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Conclusion

From the study it was concluded that the topical application of honey on the abraded surface of ileum and caecum in rabbits was effective in preventing postoperative peritoneal adhesions.

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Conflict of Interest

In honey group the incidence of adhesions was lesser compared to control group. Therefore, honey was found to have anti-adhesive property and helps in preventing post-operative intraperitoneal adhesion.

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