




Efficacy of pectin-honey hydrogel and vitamin E in prevention of post-surgical intraperitoneal adhesions in rabbits[#]

    
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Abstract

Abdominal as well as intra-visceral adhesions are considered to be one of the major post-surgical complications which is the results of inflammation, coagulation and fibrin depositions due to surgical injuries or irritations at the time of surgical intervention. In the present study, eighteen numbers of apparently healthy adult rabbits of either sex equally divided in to three groups were used to see the efficacy of Pectin-Honey Hydrogel and Olive oil-vitamin E preparation in prevention of induced intraperitoneal adhesions. Group A, Group B and Group C were treated with intraperitoneal application of normal saline, Pectin-Honey Hydrogel and Olive oil-vitamin E preparation respectively following induced peritonitis. Clinical parameters (0th, 2nd, 4th, 6th, 8th and 10th days), biochemistry of peritoneal fluid (0th, 10th, 20th and 30th days) was carried out. The surgical wounds were reopened on 10th, 20th and 30th day for macroscopic assessment of adhesions. Clinical parameters found to be significantly elevated ($P < 0.05$) on 2nd post-surgical; however, the changes were within the physiological limit. Peritoneal fluid biochemistry revealed significant higher total protein concentration in Group A followed by Group C & B. A significantly lower catalase activity of peritoneal fluid was found in Group B post-surgically. Macroscopic peritoneal and visceral adhesion score was recorded highest in Group A and lowest in Group B. The present study revealed that Pectin-Honey Hydrogel and Olive oil-vitamin E preparation effectively prevent peritoneal adhesion in rabbits and promises its clinical use.

Keywords: Pectin honey hydrogel, vitamin E, adhesion, rabbits

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Abdominal wall as well as intra-abdominal adhesions results from inflammation and coagulation processes, induced by surgery, injuries or irritation which usually damage the monolayer of cells on the basement membrane in tissues, leading to fibrin deposition. Decline in fibrinolytic activity and deposition of organized extracellular matrix (ECM) finally result in development of adhesion (Capella-Monsonis *et al.*, 2019). The incidence of formation of bridges of peritoneal tissues due to injuries of two intimate contact surfaces are recorded as high as 93% in population undergoing any surgical procedure (Takagi *et al.*, 2013). Studies on the pathogenesis and preventive strategies are lacking in spite of the severity of complications caused by post-operative adhesions. The major challenges in this regard could be the difficulty in assessing the extent and severity of adhesions for which a second surgery is required. Out of various prevention strategies adapted for prevention of peritoneal adhesions like solid barriers, fluid and gel barriers, surgical protocols, physical barriers effectively prevented formation of postoperative intra-abdominal adhesions (Hwang *et al.*, 2013). Honey, pectin and vitamin E are having several properties such as antimicrobial, anti-inflammatory, wound healing, anti-coagulant, antioxidant as well as anti-fibroblast properties that signifies their roll in prevention of formation of peritoneal adhesions (Aysan *et al.*, 2002; Carnwath *et al.*, 2014; Cooke *et al.*, 2015; Emre *et al.*, 2009; Lin *et al.*, 2013; McGee *et al.*, 1990). The present study was carried out to evaluate the roll of pectin-honey hydrogel and olive oil-vitamin E in prevention of post-surgical induced peritoneal adhesions in rabbits.

Materials and methods

Experimental design and grouping

The present study was conducted at the Department of Surgery and Radiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022 during 2021-22 and in compliance with the approval of the Institutional Animal Ethics Committee. A total of eighteen adult rabbits (*Oryctolagus cuniculus*) of either sex with in a body weight range of 1.0-2.0 kg were equally divided into three groups viz Group A, Group B

and Group C. In all the animals, standardised caecal/peritoneal abrasion was created under deep sedation using combination of Xylazine Hydrochloride (Xylaxin, Indian Immunologicals Limited) @ 5 mg/kg bw, IM and Ketamine Hydrochloride (Ketamax®-50, Troikaa Pharmaceuticals Limited) @ 35 mg/kg bw, IM. Group A animals were treated as control with intraperitoneal application of normal saline (Fig. 3); Group B animals were subjected to application of pectin-honey hydrogel (Fig. 4); while olive oil - vitamin E was applied in Group C animals (Fig. 5).



Fig. 3. Application of normal saline



Fig. 4. Application of pectin-honey hydrogel



Fig. 5. Application of Olive-oil Vitamin E



Fig. 1. Pectin-honey hydrogel

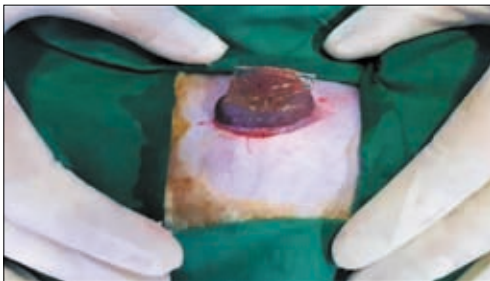


Fig. 2. Creation of abrasion

Preparations and surgical techniques

Pectin-Honey Hydrogel (Fig. 1) was prepared as per the method described by Giusto *et al.* (2016). Olive oil-vitamin E preparation was constituted by dissolving 10 mg of vitamin E (Evion®, Procter & Gamble Health Ltd) in 5 ml of Olive oil (Olive Oil, Sisco Research Laboratories Pvt. Ltd) as per the methods described by Portilla *et al.* (2004) and Yetkin *et al.* (2009). After sedation, in each rabbit approximately 4 cm long caudal ventral midline incision was made in the abdominal wall and the ileum, caecum and peritoneum were exposed and the apex of caecum was abraded

by scrapping with surgical blade (Fig. 2). The treatment regimens were applied as mentioned earlier.

Parameters and statistical analysis

Clinical parameters *viz.* rectal temperature (°F), heart rate (beat/minute), respiration rate (rate/minute) were recorded on 0th, 2nd, 4th, 6th, 8th and 10th day. The biochemistry of peritoneal fluid was carried out on 0th, 10th, 20th and 30th post-operative days by performing laparotomy in all the animals to measure the protein concentration (g/dl) and catalase activity (U/mg protein) of the peritoneal fluid as well as to record the macroscopic assessment of adhesions and the extent of adhesion as per the method described by Blauer and Collins (1988). Statistical analysis was done as per the method described by Snedecor and Cochran (1989) with the help of Statistical Analysis System 9.3 (SAS 9.3).

Results and discussion

The changes in the clinical parameters are depicted in Table 1. Non-significant as well as significant elevation in mean rectal temperature, heart rate and respiration rate were recorded in all the groups on 2nd day following surgery; however, the changes were within physiological limit and returned to normal towards the end of the study period. The changes of clinical parameters might be due to pain and inflammation provoked by surgical trauma. Author's findings were in accordance with the findings of Babu *et al.* (2014) and Konwar *et al.* (2019) in rabbits.

Table 1. Observations on clinical parameters (Mean ± SE) n=6

Parameter	Groups	Days of intervals					
		0 th	2 nd	4 th	6 th	8 th	10 th
Rectal Temperature (°F)	A	102.60 ^{ab} ±0.26	102.98 ^a ±0.21	103.30 ^a ±0.26	102.28 ^{ab} ±0.61	101.70 ^b ±0.70	103.25 ^a ±0.19
	B	102.88 ^a ±0.25	102.92 ^a ±0.59	102.68 ^a ±0.29	101.88 ^a ±0.56	102.53 ^a ±0.42	102.47 ^a ±0.41
	C	102.60 ^{ab} ±0.34	103.13 ^a ±0.20	103.33 ^a ±0.18	103.13 ^a ±0.22	101.75 ^b ±0.33	102.38 ^{ab} ±0.49
Heart Rate (Beat/Minute)	A	199.00 ^a ±5.85	237.50 ^b ±6.92	253.00 ^{bc} ±7.02	265.67 ^a ±7.90	268.50 ^a ±7.72	244.83 ^b ±15.92
	B	202.67 ^a ±5.46	232.83 ^{bd} ±5.57	257.50 ^{bc} ±4.83	257.00 ^{bc} ±9.60	261.50 ^a ±9.01	228.33 ^a ±9.36
	C	214.00 ^a ±7.45	256.00 ^b ±7.83	260.00 ^b ±6.87	255.33 ^b ±11.30	257.33 ^b ±7.02	243.33 ^b ±14.05
Respiration Rate (Rate/Minute)	A	60.17 ^a ±1.54	76.33 ^b ±2.86	72.33 ^b ±3.77	61.67 ^{ac} ±2.72	58.83 ^{ac} ±1.87	59.83 ^{ac} ±1.64
	B	58.17 ^a ±2.06	71.00 ^b ±2.90	72.00 ^b ±1.86	63.00 ^{ac} ±1.53	59.67 ^{ac} ±1.20	56.67 ^a ±1.33
	C	59.33 ^{ad} ±1.38	70.00 ^{bc} ±1.39	72.33 ^b ±1.41	64.83 ^{ac} ±1.60	57.33 ^d ±0.99	54.67 ^d ±2.04

The means with different superscripts within a row differs significantly at 5% significance level.

The changes recorded in the peritoneal fluid biochemistry are depicted in Table 2. There was significant elevation of mean total protein concentration of peritoneal fluid in all the groups on 10th post-surgical day, thereafter, a non-significant reduction was observed on 20th & 30th day in Group A and Group B; while Group C revealed significant reduction during the same days. The protein concentration of peritoneal fluid was found to be significantly higher in Group A as compared to Group B & C on 10th and 20th day observation. Development of rapid post-surgical peritoneal inflammatory reaction and well as handling of the abdominal viscera might be attributed to rise in total protein concentration in all the groups. Similar observation was also recorded by Hanson *et al.* (1992) and Shimanuki *et al.* (1986) in rabbits after abrasion of peritoneum. The higher values of total protein concentration in peritoneal fluid in Group A, when compared to Group B & C, on 10th and 20th day post-surgery may be due to application of anti-adhesive treatment in those groups which reduces inflammation. Konwar *et al.* (2019) recorded higher total protein concentration of peritoneal fluid in control group than the intraperitoneal honey group after induction of adhesion. The catalase activity of peritoneal fluid increased significantly in all the groups on 10th post-surgical day. The values showed a declining trend significantly in Group B & C, but non-significant decline was observed in Group A on 20th day. Elevated catalase activity of peritoneal fluid on 10th post-surgical day might be due to increase in oxidative stress and production of reactive oxygen species (ROS). In Group B where pectin-honey hydrogel was applied intraperitoneally, the catalase activity was markedly low on 20th day of observation as compared to the other groups, which might be due less inflammation and reactive oxygen species (ROS) production. The author's findings were in accordance with Yuzbasioglu *et al.* (2009) in honey treated rat model.

Following surgery, all the animals were closely observed to record the macroscopic changes. The signs of mild to moderate anorexia, lethargy and inactiveness for about 2-3 days were observed in all the animals, followed by a gradual recovery. Moderate anorexia, lethargy and inactiveness for about

two to three days after surgery can be attributed to surgical trauma and inflammation as reported by Singh *et al.* (2008) and Babu *et al.* (2014) in rabbits. There were no signs of prolonged inflammatory reactions, chronic discomfort, scar development, suture sinuses, wound infection, wound dehiscence, or herniation *etc.* in any of the animals during entire experimental period. The cutaneous wounds healed without complications in all the animals by the 10th post-operative day and the sutures were removed. Evidence of pain, warmth, exudation, or oedema at the surgical site after removal of sutures were not noticed. On the 10th, 20th, and 30th post-operative day, the peritoneal cavity of the animals was reopened to record grade, severity and extent of adhesions if any. The Mean+SE values of degree of intraperitoneal adhesion score (0-4) in Group A, Group B and Group C on different days are depicted in Table 3. The highest adhesion was recorded in Group A, followed by Group C and least in Group B. Intraperitoneal adhesion was seen in all the animals of Group A on day 10 (Fig. 6) and significant adhesion bands were seen between the viscera on 30th day (Fig. 7).



Fig. 6. Group A, Day 10th



Fig. 7. Group A, Day 30th

Table 2. Observations on biochemistry of peritoneal fluid (Mean \pm SE) n=6

Biochemical parameters	Groups	Days of intervals			
		0 th	10 th	20 th	30 th
Total Protein (g/dl)	A	2.57 ^{Aa} \pm 0.06	3.13 ^{Ab} \pm 0.11	3.00 ^{Ab} \pm 0.08	2.60 ^{Aa} \pm 0.06
	B	2.55 ^{Aa} \pm 0.04	2.88 ^{Bb} \pm 0.04	2.80 ^{Bbc} \pm 0.09	2.62 ^{Aac} \pm 0.05
	C	2.50 ^{Aa} \pm 0.04	3.01 ^{ABb} \pm 0.07	2.79 ^{Bc} \pm 0.04	2.53 ^{Aa} \pm 0.05
Catalase activity (U/mg)	A	37.65 ^{Aa} \pm 0.69	52.75 ^{Bb} \pm 0.93	52.40 ^{Ab} \pm 1.60	37.45 ^{Ba} \pm 1.21
	B	37.21 ^{Aa} \pm 0.87	54.81 ^{Bb} \pm 0.97	46.85 ^{Bc} \pm 0.83	39.39 ^{Ba} \pm 0.67
	C	38.66 ^{Aa} \pm 0.68	60.65 ^{Ab} \pm 1.94	52.11 ^{Ac} \pm 1.65	39.55 ^{Ba} \pm 0.79

The means with different superscripts within a row differs significantly at 5% significance level.

Table 3. Adhesion score in different groups at different intervals (Mean \pm SE) n=6

Groups	Periods			Overall
	10 th	20 th	30 th	
Group A	2.17 ^A \pm 0.17	2.83 ^A \pm 0.31	2.83 ^A \pm 0.31	2.61 ^A \pm 0.16
Group B	0.50 ^B \pm 0.22	0.67 ^B \pm 0.21	0.67 ^B \pm 0.21	0.61 ^B \pm 0.12
Group C	0.83 ^B \pm 0.17	1.00 ^B \pm 0.26	1.17 ^B \pm 0.31	1.00 ^B \pm 0.14

The means with different superscripts within a row differs significantly at 5% significance level.

**Fig. 8.** Group B, Day 10th**Fig. 9.** Group B, Day 30th**Fig. 10.** Group C, Day 10th**Fig. 11.** Group C, Day 30th

In Group B, no noticeable adhesion was recorded on 10th day (Fig. 8) and one animal showed grade 1 adhesion on 30th day (Fig. 9). Group C animals revealed mild adhesion mostly between the abdominal wall and caecum on

10th day (Fig. 10) which was on 30th day of observation (Fig. 11). Similar observations were also made by Aysan *et al.* (2002) and Konwar *et al.* (2019) on 10th post-operative day when honey was applied in injured caecum in rats. In

intraperitoneal pectin-honey hydrogel group, significantly lower adhesion score might be due to the barrier action of honey that effectively separate traumatised surfaces after surgical manipulation and reduce the extent as well as severity of adhesion. Similar observation also recorded by Saber (2010); Negahi *et al.* (2019) and Giusto *et al.* (2016) in rabbits and rat model.

Conclusion

From the present study, it may be concluded that there was non-significant variation of clinical parameters between the groups following creation of peritoneal defects. Intraperitoneal application of both Pectin-Honey Hydrogel and Olive oil-Vitamin E preparation was found effective in preventing post-operative intraperitoneal adhesions. Pectin-Honey Hydrogel (PHH) showed more promising result compared to Olive oil-Vitamin E preparation.

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Conflict of interest

The authors declared that they have no any conflict of interest.

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