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Surgical and Histopathological Evaluation of Suture Materials for Closure of Colonic Wounds after Experimental Typhlectomy in Dogs

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ABSTRACT

Typhlectomy has been prescribed for the treatment of cecal affections in dogs. The effect of suture materials on healing process after typhlectomy has not been studied yet. Twenty seven Mongrel dogs were randomly divided into 3 groups. Chromic catgut, silk and vicryl were used for groups 1, 2 and 3, respectively and 3 dogs each were euthanized on the 7th, 14th and 21st POD for evaluation of the healing process of colonic wounds. None of the animals had post-operative abnormal clinical signs. Non-significant differences in the mean height of the ridges were observed among the 3 groups. Groups 1 and 2 showed a moderate to severe mucosal necrosis at free part of inverted colonic edges, minimal cellular infiltration in lamina properia, inflammatory cell infiltration in tunica muscularis and moderate to severe tissue reaction around the suture material whereas, group 3 exhibited least mucosa necrosis, minimal destruction of tunica muscularis and low inflammatory cell reaction around suture material. Scores of these lesions did not differed significantly among the 3 groups but tissue reaction around the suture material differed significantly ($p<0.05$). In conclusion, vicryl was the most convenient and superior suture material for colonic wound healing after typhlectomy in dogs.

Key words: Colonic wound, healing, histopathology, suture material, typhlectomy

INTRODUCTION

Typhlectomy, ablation of the cecum, is indicated for many cecal diseases in large and small animals. Impaction (Wells *et al*., 1995), perforation, dilatation, inversion (Clark and Wise, 1994), intussusceptions and neoplasia (Kapatkin *et al*., 1992) are most common affections of the dog's cecum. Typhlectomy using inverting Parker-Kerr technique justifies inversion of the colonic stump thereby provides serosa to serosa apposition. Double-layer suture pattern has been advocated for minimizing the leakage of luminal contents whereas, serosa-to-serosa apposition has been recommended for preventing adhesion formation (Dean and Robertson, 1985; Ellison, 2011).

The factors responsible for smooth recovery or occurrence of post-surgical complications are; suture pattern, type of suture material, surgeon' skills, antiseptic preparation of surgical site and post-operative management. The proper suture material for enteric surgery should be decrease irritation of the intestinal wall, prevent leakage of intestinal contents and prevent adhesion formation (Orsher and Rosin, 1993). An intestinal suture line has a special importance because of the powerful movement across it, yet the healing wound must not leak or contract. Surgical management of such a wound requires a detailed knowledge of epithelialization, protein synthesis, biology of wound contraction, the surrounding milieu of the wound healing, the ground substance and the factors which alter healing (Hardy, 1990a, b). One of the most important factors implicated in the quality of the intestinal wound healing is the suture material.

Non-absorbable suture materials are advantageous for the reason that they maintain tensile strength and elicit little tissue reaction (Greenwald *et al*., 1994) but braided nature of silk threads allows surface debris and bacterial accumulation, resulting in inflammation at the surgical site surrounding the suture line (Grabb, 1991). On the other hand, absorbable suture materials incite varying degrees of tissue response due to their degradation by hydrolysis, enzymatic digestion or phagocytosis but they rapidly loss their tensile strength so they can not considered as reliable sole support to the sutured wound edges. Other factors such as handling and the desired results/outcome of surgery should also be borne in mind while selecting suture material (Fontaine and Dudley, 1978).

The ideal suture material for colonic wound stump incisions has not been studied yet; therefore, the present study was aimed to investigate different absorbable and non-absorbable suture material for efficiency and suitability to close the colonic wounds after experimental typhlectomy in dogs.

MATERIALS AND METHODS

Experiment design: Twenty seven male Mongrel dogs (30-35 kg b.wt.) were randomly allotted to constitute 3 groups (1, 2 and 3) having 9 animals each. All dogs were premedicated with atropine sulphate (0.04 mg kg^{-1}, I.M) and xylazine hydrochloride (2 mg kg^{-1}, I.M). General anesthesia is achieved by 5% thiopental sodium (10 mg kg^{-1}, I.V.). Typhlectomy was performed as per standard aseptic procedure as described by Slatter (2003). The colonic wounds in dogs from group 1, 2 and 3 were sutured with 3/0 chromic catgut, 3/0 non-absorbable braided silk and 3/0 polyglactic acid 910 (polyglactin/Vicryl), respectively. Omentalization of the colonic wound was performed as per standard procedures. Post-operative dressing of surgical wounds along with antibiotic and anti-inflammatory coverage was given for 3 Post-Operative Days (POD). On the 7th, 14th and 21st POD, three dogs from each group were euthanized by intravenous injection of an overdose of thiopental sodium 5% for studying the gross and histopathological parameters. All the animals were handled, maintained and disposed according to the experimental animal ethics approved by South Valley University, Qena, Egypt.

Clinical examination: Rectal temperature, respiratory and heart rates of each dog were recorded before surgery and also for 3 successive POD. Abdominal skin sutures were also observed daily for wound dehiscence, edema or swelling and suppuration for 7 successive POD.

Gross examination of the colonic wound: The colonic wound adhesions with omentum and other tissues such as mesenteric lymph nodes and other parts of the intestine were grossly assessed and graded using a standard scale from 1-3; one for mild adhesion at one point of the wound with a fibrous band. Two for a moderate adhesion occurred at more than one point and 3 for severe adhesion almost circumscribed entire colonic closure. Moreover, the colonic wound stump was investigated for dehiscence, leakage and presence of suture material. The height and length of the mucosal ridges, formed at the site of the colonic wound closure and the time of their disappearance as well as the presence of suture material were also recorded.

Microscopical examination of the colonic wound: Tissue specimens were obtained from the junction of the colonic wound and immersed in 10% neutral formalin for fixation. The tissue specimens were processed as per standard histopathological procedures and embedded in paraffin wax. Sections of 5 μ thickness were prepared and stained by haematoxylin and eosin. Organization of wounds was studied by staining tissue sections with Van Gieson stain. The following histopathological changes were studied, scored and recorded in all animals (Table 1):

Table 1: Description and scoring of histopathological changes of the colonic wound healing

Histopathological scoring	Description
Necrosis	
0	Intact epithelium
1	Slight necrosis
2	Moderate necrosis
3	Severe necrosis
Wound discontinuity* (Epithelialisation)**	
0	Complete apposition
1	Minimal apposition
2	Incomplete apposition
Cellular infiltration in lamina propria	
0	No infiltration
1	Minimal infiltration
2	Moderate
3	Severe
Destruction in tunica muscularis	
0	No destruction
1	Disarrangement of few muscle fibers with focal inflammation of tunica muscularis
2	Multifocal areas of inflammation of tunica muscularis
3	Extensive area of sever necrosis and cellular infiltration
Tissue reactions	
1	Thin layer of mild fibroblastic proliferation with minimal cellular infiltration
2	Mild small cellular granulomas composed of suture material shreds surrounded by moderate inflammatory cells
3	Large granulomas composed of central suture material with necrosis, numerous cellular infiltrations with fibroblastic capsule and the surrounding tissue was severely destructed
Presence/absence of suture materials	
0	Absence of the suture materials
1	Tiny shreds of suture materials
2	Remnant of suture materials
3	Few intact threads of suture materials

*Degree of apposition of the colonic edge, **Degree of integrity of the epithelium overlying the colonic wound

- Tunica mucosa: Necrosis, epithelialisation and cellular infiltration were assessed and scored
- Tunica submucosa: Thickening of the submucosal layer, lymphoglandular changes, fibroblast proliferation and newly-formed blood vessels were evaluated
- Tunica muscularis: Destruction and inflammation in muscular layer of colonic stump were examined
- Tunica serosa: Presence of suture materials and adhesions of serosa were assessed
- Tissue reactions including inflammatory cell infiltration and fibroblasts proliferation around the suture materials were evaluated

Statistical analysis: The severity of adhesions between the colonic wound and omentum and the histopathological examination was statistically analyzed between groups by using the survival (event) analysis test. Mean height and length of ridges were subjected to test of significance by one-way ANOVA wherein, mean values of height and length of ridges among all groups were compared by Dunnett's test using Graphed Pad Prism (Graphed Pad software, San Diego, CA, USA). Results were considered significant where $p<0.05$.

RESULTS

All animals after typhlectomy were survived and had no abnormal signs from the 2nd POD till the end of the experiment. The mean of the temperature, respiratory and heart rates were 38.6±0.55°C, 39±8 and 106±16 min^{-1}, respectively.

Gross findings: The degree of adhesion of the colonic wound to the omentum varied among the 3 groups. Almost circumscribed severe adhesions were observed in animals from group 1. Moderate adhesions, more than one point, were observed in animals of group 2 whereas, mild adhesions, almost one point, were observed in animals from group 3. The statistical analysis revealed highest degree of surgical wound adhesion with omentum in animals from group 1 followed by those of group 2 and 3 in a decreasing order, however, the differences were statistically non-significant (Fig. 1). Similarly, non-significant differences in adhesions with the surrounding tissues were observed among the groups wherein, highest prevalence of adhesions was in animals of group 1 and 2 as compared with those of group 3 (Table 2). Wound dehiscence was not observed in all groups except one dog in group 1 at the 7th POD. Presence of suture material was recorded almost on the serosal surface in the 3 groups.

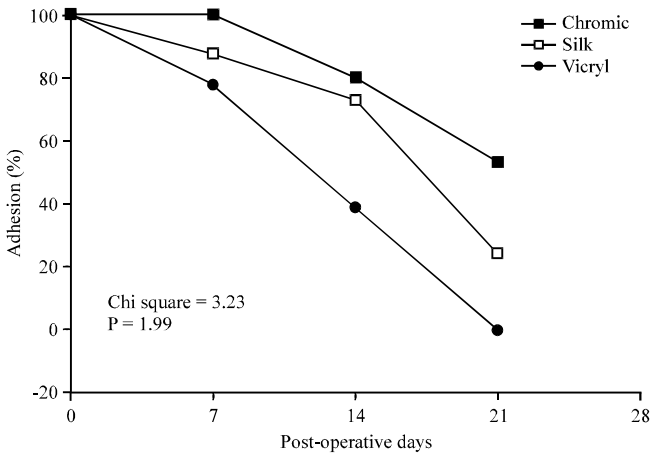

Fig. 1: Histogram illustrating adhesion of the colonic stump to the omentum and other tissues

Table 2: Prevalence of adhesion of colonic wound to intestine and other tissues using 3 types of suture material at the 7th, 14th and 21st POD

	Group 1			Group 2			Group 3		
Adhesions	7th	14th	21st	7th	14th	21st	7th	14th	21st
Adhesion with mesenteric lymph node	1/3	0/3	1/3	2/3	1/3	1/3	2/3	0/3	2/3
Adhesion with l. node and intestines	2/3	2/3	2/3	0/3	0/3	0/3	0/3	0/3	0/3
Adhesion with intestines	0/3	1/3	0/3	0/3	1/3	1/3	1/3	0/3	0/3

Table 3: Mean height and length (mm) of the mucosal ridge (±SD) after colonic wound closure using 3 different suture materials

	Group 1 (Catgut)		Group 2 (Silk)		Group 3 (Vicryl)	
Euthanasia	Height	Length	Height	Length	Height	Length
POD						
7th	6.33±2.07	9.32±0.98[a]	4.61±0.89	8.4±1.390	5.42±1.67	7.8±1.66[a]
14th	4.98±2.04	8.40±0.52[a]	3.49±1.44	7.2±1.200	4.27±2.23	7.98±1.0[a]
21st	3.83±1.55	6.85±0.60[b]	3.93±1.67	9.38±0.54	3.36±0.83	4.46±1.2[b]

The mean and standard deviation values of heights and lengths of the colonic mucosal ridges have been depicted in Table 3. Mean heights of the ridges among all groups and also at different POD did not differ significantly. The mean length of mucosal ridges in animals of group 2 also did not differ significantly throughout the experiment but in animals of groups 1 and 3, the length of the ridges at the 21st POD was significantly lower than those at the 7th and 14th POD. The remnant of the suture materials on the mucosal surface of the colonic wound were seen in group 2 but not in group 1 and 3.

Microscopic findings: At the 7th POD, the mucosal fold was severely necrosed with less epithelisation in group 1 and 2 (Fig. 2a). Moreover, the tunica muscularis was moderately to severely destroyed, where extensive areas of severe necrosis and inflammatory cell infiltration between muscle fibers were observed. The suture material was surrounded by large granulomas composed of numerous cellular infiltrations with fibroblastic capsule and the surrounding tissue was severely destroyed. However, a mild to moderate mucosal necrosis with mild small cellular granulomas composed of moderate inflammatory cells, in addition to mild destruction to the tunica muscularis, were seen in group 3.

At the 14th POD, a moderate degree of mucosal necrosis with active process of epithelisation (Fig. 2b) and multifocal areas of inflamed tunica muscularis were observed in groups 1 and 2. Moderate to severe tissue reaction around the suture materials was also observed (Fig. 2d, e). In contrast, animals of group 3 revealed minimal mucosal necrosis with complete epithelialisation of the mucosa on the colonic wound (Fig. 2b, c). Thin layer of mild fibroblastic proliferation with minimal cellular infiltration was observed around the suture material (Fig. 2f). The destruction incurred to tunica muscularis was mild.

At the 21st POD, intact mucosal layer with good epithelisation and minimal cellular infiltration in the lamina properia was observed. Remnant of suture material surrounded by large cellular granulomatous and fibrous connective tissue with destruction of the tunica muscularis was observed in groups 1 and 2 (Fig. 2g, h). The destruction of tunica muscularis was subsided at this stage. In group 3, apposition with complete epithelialisation and minimal cellular infiltration at the wound edges of the mucosal layer was observed. The destruction of tunica muscularis and the tissue reaction were almost minimal at this stage (Fig. 2i).

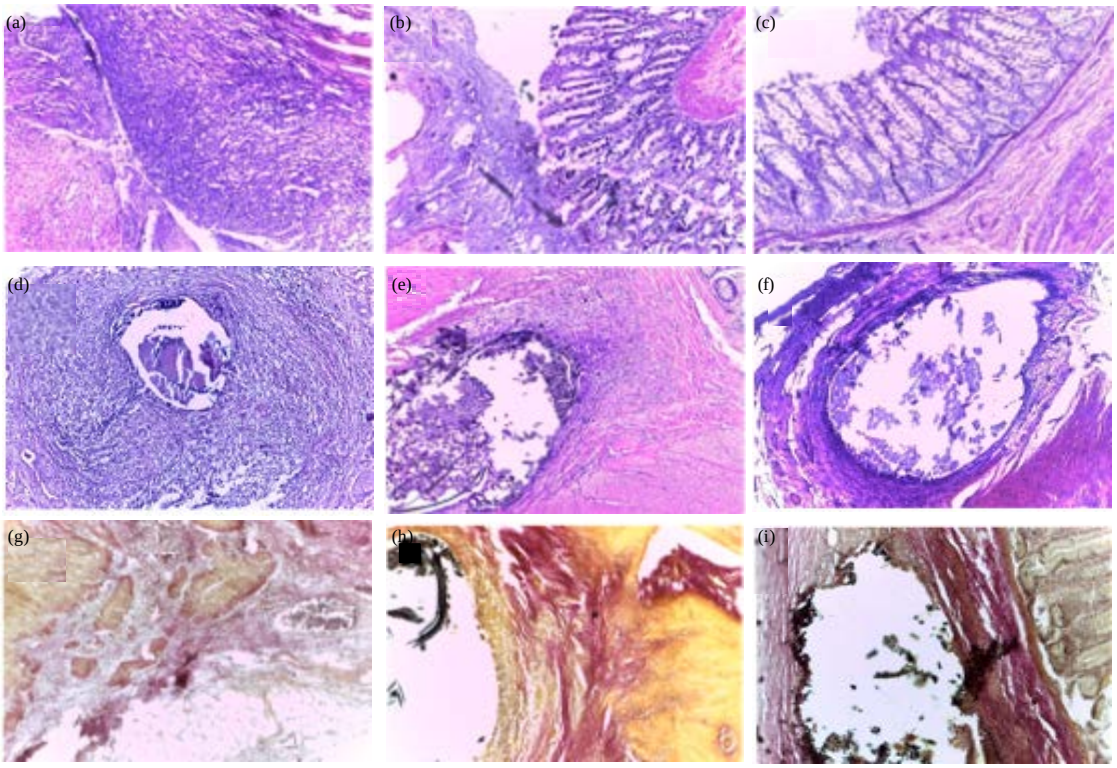

Fig. 2(a-i): (a) Mucosal necrosis and incomplete apposition of wound edges with severe inflammatory cellular infiltration in submucosa were observed at 7th POD in group 1 (H and E, 4X, 10X, 20X), (b) Severe necrosis in the mucosal fold of the free edge projection in the intestine lumen at 14th POD in group 2 compared with almost intact mucosa in the continued wound edge (H and E, 4X, 10X, 20X), (c) Almost intact mucosal fold with minimal inflammatory cell infiltration in lamina properia at 14th POD in group 3 (H and E, 4X, 10X, 20X), (d) Severe inflammatory cell infiltration mainly lymphocytes and macrophages with proliferation of fibroblast was observed around the remnant of the suture material at 14th POD in group 1 (H and E, 4X, 10X, 20X), (e) Tunica muscularis were severely destructed with remnant of suture materials. The latter surrounded by lymphocyte, macrophages and fibroblast proliferation at 14th POD in group 2 (H and E, 4X, 10X, 20X), (f) Threads of suture material in serosal layer with minimal cellular reaction at the 14th POD in group 3 (H and E, 4X, 10X, 20X), (g) Severe destruction of tunica muscularis replaced by fibrous connective tissue and more cellular reaction at 21st POD in group 1 (Van Giesson, X4), (h) Remnant of suture material is surrounded by cellular granuloma and fibrous connective tissue at 21st POD in group 2. Note destruction of the tunica muscularis (Van Giesson, 4X, 10X, 20X) and (i) Remnant of suture material is surrounded by minimal cellular reaction. Note the condensation of the submucosa at 21st POD in group 3 (Van Giesson, 4X, 10X, 20X)

The scores of mucosal necrosis, wound discontinuity, granulation tissue formation and destruction of tunica muscularis were non-significantly different among the 3 groups whereas, tissue reaction around suture materials was significantly ($p<0.05$) higher in group 1 as compared

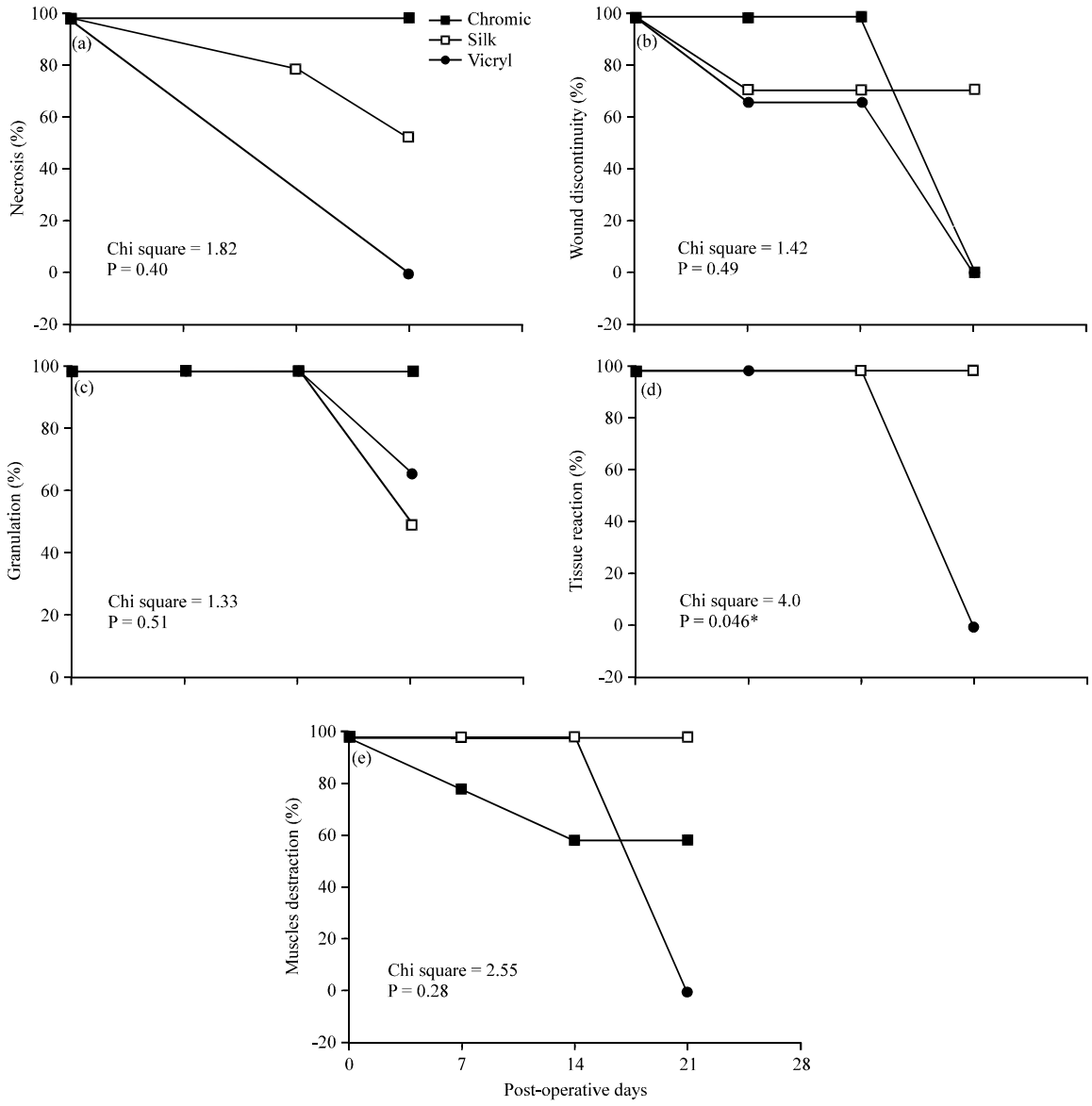

Fig. 3(a-e): Statistical analysis of the scores of the histopathological picture of the colonic wound healing after using three types of suture materials. *Significant difference ($p<0.05$)

to that in group 2 and 3 in a decreasing order. Minimal necrosis, destruction of tunica muscularis and granulation tissue formation with good epithelialisation were observed in group 3 (Fig. 3).

DISCUSSION

Surgery of the large intestine is associated with many complications such as wound dehiscence, leakage, adhesions and peritonitis (Borenstein *et al.*, 2001). The cecum, as a part of the large intestine, may also have the same complications during its surgery. In the present study, silk was selected as a good example for a natural non absorbable suture material. Whereas, chromic catgut and vicryl as natural and synthetic absorbable suture materials, respectively were investigated to

evaluate the most suitable/preferred suture material for closing colonic wound during typhlectomy. Therefore, the operation was performed by one surgeon and one assistant and both of them were comprehensively trained on typhlectomy before starting the experiment.

Hedlund (2002) and Brown (2003) recommended omentalisation immediately after performing intestinal surgery for prevention of leakage of intestinal chime. Therefore, the adhesions of the colonic wound to the omentum observed in the present study are preferred outcomes rather than a post-operative complication because omentalisation was applied during the surgical operation.

Wound dehiscence might play a second role in adhesions formation (Crowe and Bjorling, 1993), but in the present study, only one dog exhibited post-surgical colonic wound dehiscence. Therefore, adhesions observed in other dogs might be attributed to roughening of the serosal surface caused by presence of suture materials thereon. In the present study, severe adhesions were observed in dogs where chromic catgut and silk were used for closing colonic wound in groups 1 and 2, however, mild to moderate adhesions were observed in group 3 when using vicryl for wound closure. These findings are in agreement with those reported by Bennett (1988) and Akinrinmade and Lawal (2010).

In the present study, remnants of silk were found on the mucosa of the colon at the 21st POD that may result in permanent intra-luminal ridge formation, but this hypothesis requires further extensive study for confirmation. Moreover, presence of silk remnant on the mucosal surface of the colonic wound indicates that the healing process go towards extrusion of suture material rather than capsulation that in turn may lead to ulceration around the intra-luminal hanged suture material which may act as a nidus for fecal matter deposition and subsequent faecolith formation which could lead to intestinal obstruction (Pecha *et al*., 1998).

Dogs in group 3 showed a minimal necrosis in the mucosa, complete apposition of the wound edges with complete epithelization of the mucosal layer, regenerated submucosa and minimal cellular infiltrations surrounding the remnant of suture materials at the serosal layer. However, dogs in groups 1 and 2 have moderate to severe necrosis of the mucosal folds with partial epithelization, slightly thickened submucosa with granulation tissue related to the wound edges and a severe inflammatory cell reaction surrounding remnant threads of the suture materials in serosa. The healing process and the inflammatory reacation observed around the remanats of suture materials corroborates with the findings reported by Bennett (1988) and Bellenger (1982).

Chromic catgut is digested or phagocytosed by cellular elements in the blood that leads to engorgement of blood vessels at the site of healing with subsequent more tissue reaction (Greenwald *et al*., 1994). Silk threads act as a foreign material thereby incites encapsulation (Grabb, 1991) whereas Vicryl has been reported to get hydrolyzed into natural body tissues metabolites (Yaltirik *et al*., 2003).

On the basis of clinical, surgical and histopathological evaluation of silk, catgut and Vicryl for closing the colon wound during typhlectomy, Vicryl was found to be the most suitable suture material because it bears sufficient tensile strength required for closure of colonic wound. In addition, it causes least necrosis and better epithelization, mild to moderate adhesions with surrounding tissues and minimal cellular infiltration/tissue reaction as compared with silk and catgut.

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