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EVALUATION OF INTRAPERITONEAL USE OF SODIUM CARBOXYMETHYLCELLULOSE SOLUTION FOR PREVENTION OF EXPERIMENTALLY INDUCED ABDOMINAL ADHESIONS IN DONKEYS

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SUMMARY

The present study was conducted to evaluate the effect of intraperitoneal use of sodium carboxymethylcellulose solution (1% & 2%) in prevention of experimentally induced abdominal adhesion. 15 healthy adult donkeys were allocated in 3 equal groups each of 5 animals. Group I kept as control while group II & III used as treated groups. In control group, donkeys were treated with a sterile solution of sodium chloride 0.9 % intraperitoneally. In treatment groups, donkeys were treated with a sterile solution of sodium carboxymethylcellulose (SCMC) in a dose of 7 ml/kg of body weight intraperitoneally in concentration of 1% for group II& 2% for group III. After the end of the experimental period, histopathological examination was done to all groups. Results revealed intraabdominal adhesions associated with abraded, anastomotic sites and a distant segment of jejunum in all cases of control group. In treated donkeys with 1% SCMC solution, there were no adhesions detected in 4 donkeys but one case had adhesion between the jejunal abrasion site and the adjacent peritoneum. No adhesion was noticed at the anastomotic sites. In treated donkeys with 2% SCMC solution, all cases had ecchymotic serosal hemorrhage at abrasion sites and mesentery with developed peritonitis.

INTRODUCTION

Adhesions are formed as part of normal response to peritoneal injury and inflammation (Ellis, 1982). Adhesions become a clinical problem when they compress or anatomically distort the intestine that lead to intestinal constriction, incarceration, or volvulus, predisposing the patient to intestinal obstruction and abdominal pain. Adhesion formation is the most common cause of intestinal obstruction after small intestinal surgery and is the second most common cause for repeat-

ed celiotomy in horses with gastrointestinal disease and intestinal ischemic necrosis (Baxter, et al., 1989; Parker, et al., 1989 and Fubini, 1990). Parker, et al. (1989) added that repeated celiotomy for treatment of adhesions is consider poor prognosis and the most effective treatment is prevention.

The most effective method to prevent adhesion or at least to minimize the formation of adhesion in horses is excellent surgical technique that minimizes peritonitis (Fubini, 1990) and the use of intraperitoneal administration of high molecular weight solutions such as sodium carboxymethylecellulose in horses (Moll, et al., 1991; Mueller, et al., 1995 ; Mueller, et al., 2000 a & b; Hay, et al., 2001; Murphy, et al., 2002 and Eggleston, et al., 2004).

The objective of this study is based on evaluation the effect of intraperitoneal use of 1% & 2% of sodium carboxymethylcellulose (SCMC) to prevent or at least minimize experimentally induced abdominal adhesions formation by application of serosal jejunal trauma and jejunal resection and anastomosis in donkeys.

MATERIALS AND METHODS

This work was carried out on 15 healthy adult donkeys weighing between 175 to 225 kg of different ages ranging from 3 to 5 years old. All animals were apparently clinically healthy. Animals were maintained prior to start of the experiment in a special house at the Surgery Department, Faculty of Veterinary Medicine, Suez Canal University. Donkeys were divided into:

I- Control group:

In this group, five donkeys were treated with a sterile solution of sodium chloride 0.9 % intraperitoneally after induction of adhesion.

II- Treatment group with 1% of SCMC:

In this group, five donkeys were treated with a sterile solution of 1% sodium carboxymethylcellulose (SCMC) in a dose of 7 ml/kg of body weight intraperitoneally after induction of adhesion.

III- Treatment group with 2% of SCMC:

As previous group but donkeys were treated with a sterile solution of 2 % SCMC.

Preparation of SCMC

A 1% solution of SCMC was prepared by boiling 200 ml of sterile water and adding 10g of SCMC powder (El-Nasr Pharmaceutical Chemicals co. Abu Zaabal, Egypt) while stirring. After the SCMC was in solution, additional sterile water was added while stirring to bring the total volume to one litre. The SCMC solution was then transferred into glass bottle and autoclaved at 121°C for 20 minutes.

Technique of operation:

Food was withheld from donkeys for 12 hours before surgery. Animals were premedicated by

intramuscular injection of chloropromazine hydrochloride (Neurazine; Misr co. Pharm. Ind. S.A.A, Egypt) in a dose of 1 mg / kg body weight (Hall, et al., 2001). Site of operation is prepared via clipping and shaving then disinfected with povidone iodine solution (Betadine: Povidone- Iodine U.S.P. 10% W/V Betaderm 20 gm, El Nile-Co.). Surgery was run under the effect of IV infusion of 10 % chloral hydrate solution (Chloral hydrate: Schutz & Co (CmbH & Co) Hamburg) in a dose of 5 gm / 50 kg body weight then 10 mg / kg B.W. of 5 % thiopental sodium solution (Thiopental sodium: Egyptian International Pharm. Industries Company, A. R. E) (Hall, et al., 2001). Donkeys were positioned in dorsal recumbency and the area of operation was prepared aseptically and draped with sterile towels fixed in position with sterile towel clamps. Sodium chloride 0.9% (10 ml/kg/h IV) (El-Nasr Pharmaceutical Chemicals co., Egypt) was administered during the surgical procedure. Ventral median laparotomy incisions were made with length about 30-40 cm then abdominal explorations were performed to examine the viscerae. The jejunum was exteriorized and 500 ml of 1% or 2% SCMC was used to lubricate the small intestine during our manipulations in the treatment groups, whereas an equal volume of a sterile sodium chloride 0.9 % solution was used to lubricate the small intestine in the control group.

The effect of SCMC on postoperative adhesion formation was evaluated using an established model of serosal trauma according to (Moll, et al., 1991) to induce intra-abdominal adhesions. Two areas of the antimesenteric border of the jejunum were briskly rubbed with a sterile dry gauze, and then two simple interrupted sutures using 2-0 chromic catgut that did not penetrate the intestinal lumen were placed in each abraded area. Moreover, jejunal resection and end-to-end jejunal anastomoses were performed. The anastomoses were closed in 2 layers: a simple interrupted pattern using 2-0 polyglactin 910 (Vicryl:Manufactured Johnson & Johnson Jutl), followed by a Lembert's pattern in the seromuscular layer using 3-0 polyglactin 910. In the treatment groups, another 500 mL of 1% or 2% SCMC was applied to the intestine at the end of surgical manipulation. An equal volume of sterile sodium chloride 0.9 % solution was applied to the intestine in the control group. The linea alba was closed using polyglactin 910 No. 3 in a simple continuous pattern and subcutaneous tissue was closed with polyglactin 910 in a simple continuous pattern No. 0. Skin edges were sutured using silk No. 2 in a simple interrupted suture pattern.

Postoperative Care:

Each animal was injected with 1500 IU of antitetanic serum (Egyptian organization for biological products and vaccines, Agouza, Giza, Egypt) subcutaneously, 2 grams of amoxicillin intramuscular (E.Mox: Egyptian International Pharm. Industries Company, A. R. E) and dipyrone (Analgin 50 %: El-Nasr Pharmaceutical Chemicals co., Egypt.) in a dose of 10 mg/kg I.M. for 3 days.. After recovery, donkeys were allowed water free choice and were gradually returned to full feed over the next

48 hours. Each donkey was observed for attitude, pulse, respiratory rate, rectal temperature, signs of abdominal pain, and incision swelling or discharge. Any donkey with clinical signs of depression, inappetance, kicking of its abdomen was treated by administration of 10 mg / kg dipyrone 50% I.M. every 6 hours; 0.03 mg / kg of atropine sulphate 1 % I.M. (Egyptian Co. for Chemicals & Pharmaceuticals (ADWIA) S. A. E. 10th of Ramadan city, Egypt) and sodium chloride 0.9% (10 ml/kg/h IV).

Donkeys in group III were euthanatized using an over dose of pentobarbital sodium solution three days postoperatively due to severe deterioration in their healthy conditions, while in the other groups, donkeys were euthanatized using an overdose of pentobarbital sodium solution 15 days postoperatively. The abdominal incision, peritoneal cavity, and all abdominal viscerae were evaluated for adhesions and any other abnormalities. Tissue specimens from representative adhesions were collected for histopathologic examination.

Histopathology study:

The samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at about 4-6μ and stained with haematoxylin and eosin (Bancroft, et al., 1990).

Statistical Analysis

The frequency of intra-abdominal adhesion formation between control and treatment groups compared were analyzed by Chi square method using SAS (1989).

RESULTS

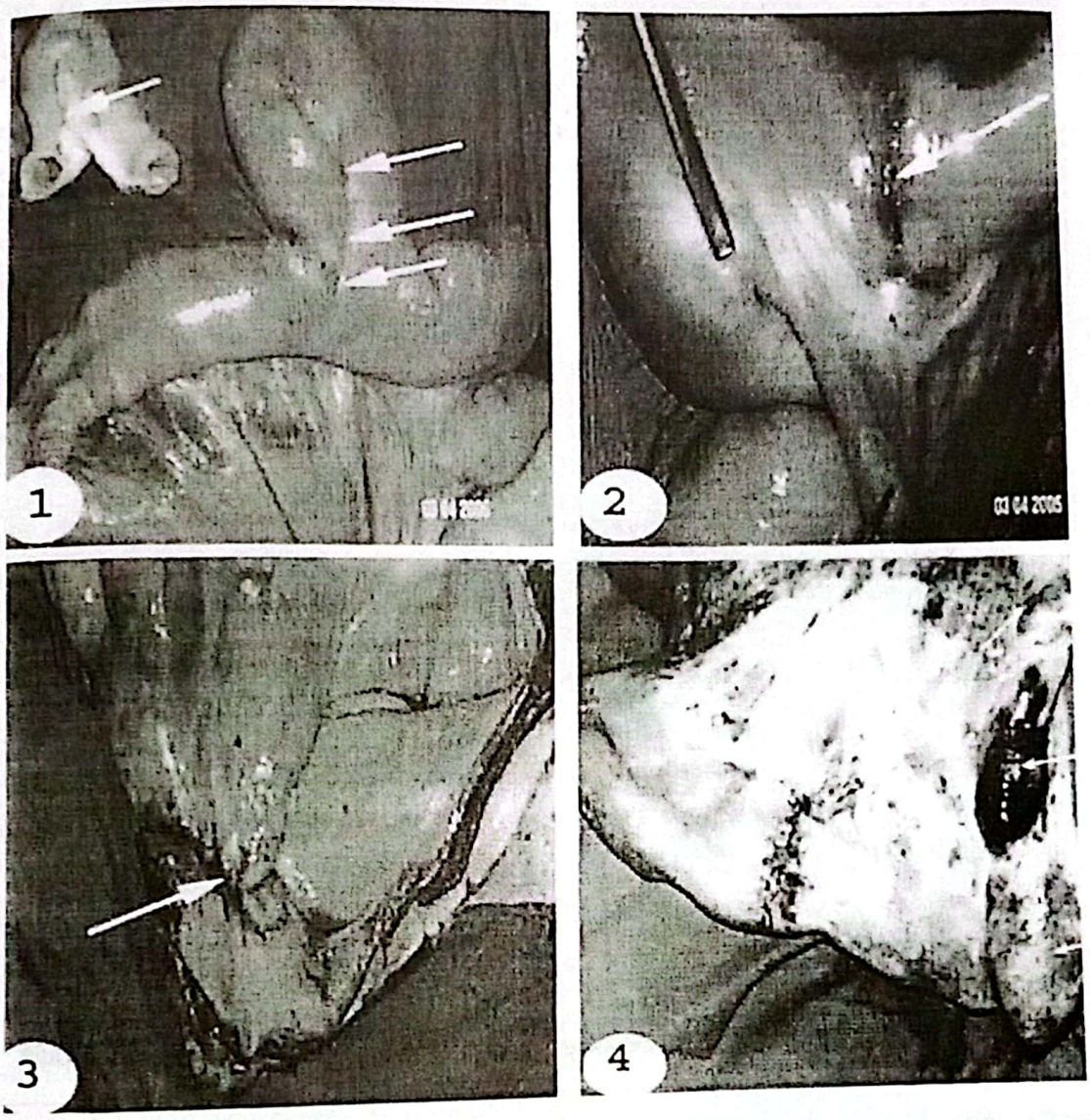
Two control donkeys and one treated donkey with 1% SCMC showed episode of postoperative abdominal pains that responded to medical treatment.

In group III treated with 2 % SCMC, all donkeys suffered from severe depression, pyrexia, anorexia, inappetance, kicking of the abdomen, straining, gastric reflux from mouth and edematous swelling discharging peritoneal fluid from the wound incision. These donkeys did not respond to medical treatment and euthanatized by an over dose of thiopental sodium 5 % after 72 hours postoperatively.

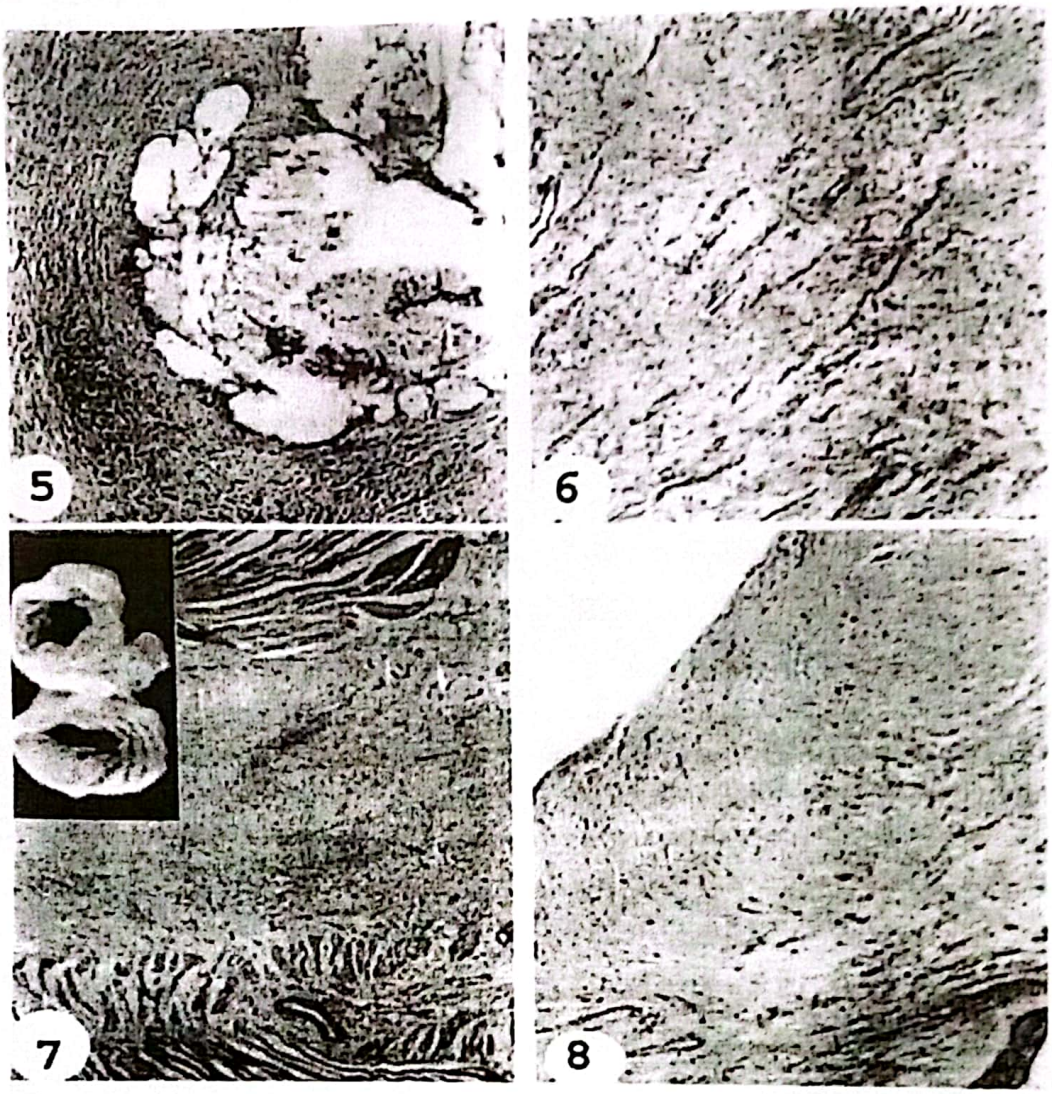
Gross pathology:

The frequency of intra-abdominal adhesion formation between control and treatment group with 1% were significantly difference ($P < 0.01$). In control group, intraabdominal adhesions were appeared in all donkeys. The jejunum at the abrasion sites was thickened with focal areas of serosal hemorrhage that associated with anastomotic site and a distant segment of jejunum (Fig 1). None of the adhesions resulted in obstruction or stricture of the jejunal lumen.

In treated donkeys with 1% SCMC solution, there were no adhesions noticed in 4 donkeys (Fig 2) while one donkey had adhesion between jejunal abrasion site and the adjacent peritoneum (Fig. 3). No adhesion was observed at the anastomotic sites.



(Fig. 1): Showing adhesion associated with anastomotic site and a distant segment of jejunum in control group (arrows).
(Fig. 2): Showing no adhesions present at anastomatic site in treated donkeys with 1 % SCMC solution (arrow).
(Fig. 3): Showing adhesion between jejunal abrasion site and the adjacent peritoneum in one donkey that treated with 1 % SCMC solution (arrow).
(Fig. 4): Showing ecchymotic hemorrhages and suffusions at serosal abrasion sites and mesentery in a donkey that treated with 2 % SCMC solution (arrows).

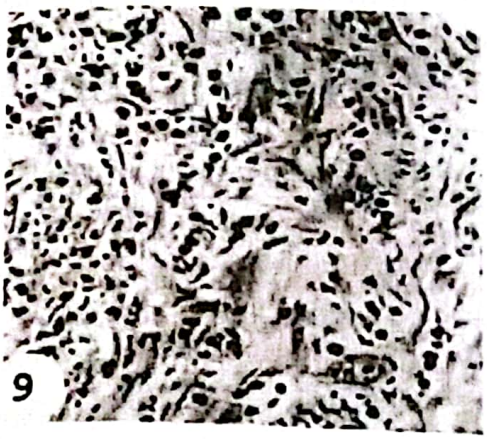


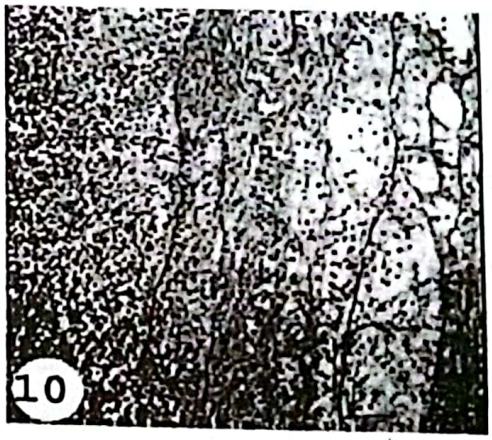
(Fig. 5): Group I, jejunum at the anastomotic site, showing intense infiltration of neutrophils and some eosinophils around the suture material, H&E. X20

(Fig. 6): Group I, the granulation tissue at the abrasion site, showing prominent capillaries, large fibroblasts, and relatively mature collagen. H&E. X 10.

(Fig. 7): Group I, adhesion formation between the abraded jejunal site and a distant segment of jejunum. It consisted of granulation tissue extending outside the outer serosal layers of the two segments. H&E. X 4.

(Fig. 8): Treated group with 1%, showing no adhesion at the serosal surface of jejunum. The outer surface of jejunum was smooth and covered with mesothelium. H&E. X 4.





(Fig. 9): Treated group with 1%, showing massive infiltration of the granulation tissue with eosinophils. H&E. X 40.
(Fig.10): Treated group with 2%, showing massive infiltration of neutrophils, eosinophils and fibrinopurellent exudates. H&E. X 40.

In treated donkeys with 2% SCMC solution, all cases had ecchymatic hemorrhages and suffusions at serosal abrasion sites and mesentery with developed peritonitis (Fig. 4).

Histopathology:

Histological sections of the jejunal abrasion and anastmosis sites from all animals of the control group were characterized by a plaque of granulation tissue adjoining with, and extending from the serosa and invading the musculosa. The plaque was centered on the suture material, and there was an intense infiltration of neutrophils and some eosinophils around the suture material (Fig. 5). Prominent capillaries, large fibroblasts, and relatively mature collagen were characterized the granulation tissue (Fig. 6). The abrasion sites and the anastomotic sites associated with adhesion formation consisted of granulation tissue extending outside the outer serosal layers of the two segments. Fibrous adhesions occurred between the abraded jejunal site and a distant segment of jejunum (Fig.7).

In treated group with 1% SCMC, the serosal surface was covered with a layer of fibrinous exudate that was covered by a layer of mesothelial cells and macrophages. The abrasion sites and the anastomotic sites showed no adhesion at the serosal surface of jejunum. The outer surface of jejunum was smooth and covered with mesothelium (Fig. 8). The granulation tissue was similar to control group but there was massive eosinophilic infiltration (Fig. 9).

In treated group with 2% SCMC, there was acute peritonitis which represented by massive infiltration of neutrophils, eosinophils and fibrinopurellent exudates (Fig.10).

DISCUSSION

Treatment of horses with abdominal pain associated with intra-abdominal adhesions is difficult and associated with poor long-term survival time (Hay and Mueller, 1998). Therefore, prevention of adhesions is important. Whereas proper aseptic, atraumatic, surgical technique is the most important factor for minimizing intra-abdominal adhesion formation. The extent of serosal or peritoneal inflammation often dictates the use of ancillary therapies directed at adhesion prevention. Identification of therapeutic agents that effectively reduce postoperative intra-abdominal adhesion formation may be beneficial in reducing the morbidity and mortality associated with equine abdominal surgical diseases (Mueller, et al., 2000 a&b).

In accordance with Moll, et al. (1991) who reported that both serosal abrasion and intestinal ischemia models had used to evaluate adhesion prevention in horses. In addition to abrasion, jejunal resection and anastomosis was done in an attempt to more closely correlate the model to a clinical situation in which anastomosis may be required. This result supported in horses by (Mueller, et al., 2000 a &b and Hay, et al., 2001).

In the present study, the dose of SCMC was chosen on the basis of previous literatures data that demonstrated an inhibitory effect of this drug on intraabdominal adhesions after intestinal resection and anastomosis in horses (Mueller, et al., 2000 a &b and Hay, et al., 2001).

In the control group, all donkeys had intra-abdominal adhesions that associated with anastomotic site and a distant segment of the jejunum. None of the adhesions resulted in obstruction or stricture of the jejunal lumen. Similar results were obtained by Mueller, et al. (2000 a & b) and Hay, et al. (2001) who mentioned that model of serosal trauma reliably induced adhesion formation without excessive morbidity or mortality.

Histological sections of the jejunal abrasion and anastmosis sites were characterized by a plaque of granulation tissue that was centered on the suture material, and there was an intense infiltration of neutrophils and some eosinophils in control group. The abrasion sites and the anastomotic sites associated with adhesion formation consisted of granulation tissue extending outside the outer serosal layers, these results came in accordance with that of (Mueller et al., 2000 a & b, Hay et al., 2001).

In this study, the use of 1 % of SCMC prevented the formation of intra-abdominal adhesions after serosal abrasions and jejunum resection and anastomosis in treated donkeys (four animals out of five). Healing without complication and no perianastomotic abscess or leakage was observed at necropsy. The abrasion sites and the anastomotic sites showed no adhesion at the serosal surface of jejunum. The outer surface of jejunum was smooth and covered with normal serous surface. This is attributed to lubricating properties of SCMC that minimized peritoneal injury and inflammation secondary to manipulation and there-

by minimized fibrin formation and deposition. This result go hand to hand with that reported by Moll, et al. (1991; Mueller, et al. (2000 a & b) and Hay, et al. (2001) who conculded that when peritoneal injury is present, SCMC is thought to aid in adhesion prevention by creating a ihydroflotation effectî that mechanically separates serosal and peritoneal surfaces in the equine abdomen. Another tenable explanation is that SCMC produces its beneficial effect by curtailing fibroblast activities or proliferation and preventing fibrin deposition on the serosal surfaces of the injury (Ryan and Sax, 1995). It may also inhibit the movement of inflammatory cells and cellular elements during the period of peritoneal repair (Hemadeh, et al., 1993 and Ryan and Sax, 1995).

Using 500 ml of 1% SCMC was sufficient to coat the entire jejunum and resulted in lubrication of the intestine and the surgeon's gloves that subjectively greatly reduced friction during intestinal manipulation. The second 500 ml of 1% SCMC, applied after manipulation, resulted in pooling of the SCMC at the surgical site. This result was agreed with that result reported in horses by Mueller, et al. (2000 a & b) and Hay, et al. (2001) who mentioned that, precoating the intestine with SCMC before manipulation decreased adhesion formation compared with application of SCMC after manipulation.

In one treated donkey with 1% of SCMC in this study, an adhesion developed to the adjacent peritoneum at one abrasion site and this is may be attributed to that SCMC at this adhesion site did not completely cover the peritoneum immediately adjacent to the abrasion site.

Treated donkeys with 2% SCMC had ecchymotic serosal hemorrhage at serosal abrasion sites and mesentery that was thought to systemic absorption of highly concentrated solution which lead to postoperative pyrexia, depression, and anorexia. Similar result was obtained in horses by (Burkhard, et al., 1996) who administered large volumes (2-4 L) of intraperitoneal SCMC. On the other hand, previous studies in ponies and horses which used 7 ml/kg of 1% SCMC have not resulted in these complications (Moll, et al., 1991 and Mueller, et al., 1995). Moreover, Hay, et al. (2001) concluded that, 4 to 6 ml/kg of 1% SCMC was effective in preventing adhesion formation, and postoperative fever, depression, and anorexia. For this reason, the potential systemic effects of high volume intraperitoneal SCMC administration should be considered before exceeding 7 ml/kg of 1% SCMC in donkeys.

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