Effect of glycerol in reduction of post-surgical adhesions in rat's model

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Abstract

This study was designed to investigate the role of glycerol in reducing of adhesions following surgery. Thirty Wistar albino male rats were randomly assigned into three groups; T 1 and T2, twelve rats for each, and six for control. Adhesion model was created in all rats. The rats in T1 were injected intraperitoneally with 5ml of glycerol 14.3%. Rats in T2 were injected intraperitoneally with 5ml of glycerol 28.6%. Rats in control groups were injected intraperitoneally with 5ml of sterile normal saline. All rats were scarified at postoperative day 14, and the gross macroscopic and microscopic score adhesions parameters were evaluated. The gross macroscopic adhesions were graded in a blind fashion, while the microscopic adhesions parameters (fibrosis, inflammation, and vascular proliferation) were gradedfrom 0-3. Adhesions were statisticallysignificantly in rats of the control group when compared to treatment groups ($p \leq 0.01$). In conclusion, this study reveals that intra peritoneal administration of glycerol (14.3%, and 28.6%) to serosal abrasions and caecotomies in rats has a role in reduction of intra-abdominal adhesion formation after surgical manipulation.

تأثير الكليسرول في اختزال الألتصاقات ما بعد المداخلات الجراحية في الجرذان

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الخلاصة

هدفت الدراسة الحالية إلى معرفة تأثير الكليسيرول في تقليل الألتصاقات ما بعد المداخلات الجراحية. استخدم للدراسة 30 جرذا ذكرا من نوع ويستر البابنو، وتم توزيعها عشوائيا إلى ثلاثة مجاميع؛ المعاملة 1 و2 والتي ضمت على 12 جرذا، أما المعاملة 3 (مجموعة السيطرة) فاحتوت على (6) جرذان. تم حقن الجرذان في المعاملة 1 و2 عن طريق التجويف الخلبي بـ (5) ملليليتر من الكليسرول 14.3% و28.6% على التوالي بعد استحداث عامل الالتصاق جراحيا في الجوف البطني. أما جرذان مجموعة السيطرة ، فقد تم حقنهما بـ (5) ملليليتر من المحلول الملحي الفسلجي بنفس الطريقة. أجريت الصفة التشريحية على الجرذان بعد 14 يوما من الجراحة لتقييم مستويات (معايير) الالتصاقات العيانية والمجهرية (التليف، الانتانات، وتكاثر الأوعية الدموية) والتي درجت بالمستويات من 0-3. أظهرت النتائج الإحصائية بان نسب حدوث الالتصاقات كانت أكثر معنويا ($p \leq 0.01$) في المعاملة 3 مقارنتا بالمعاملتين 1 و2. وتم الاستنتاج من نتائج هذه الدراسة التجريبية بان حقن التجويف الخلبي للجرذان بالكليسرول بالتراكيز 14.3% و28.6% بعد استحداث السحجات المصلية وبضع القولون فيها، له دور معنوي في اختزال تكوين الالتصاقات الخلبية ما بعد التداخلات الجراحية.

Introduction

Adhesions are connective tissue bridge or internal "scars" that are formed after trauma involving the peritoneum and the injured tissue surface or directly between tissue surfaces (1). When the peritoneum is injured by trauma, ischemia, inflammation, thermal, foreign body reaction or infection, the resulting bleeding and leakage of plasma proteins form damaged surfaces forms a fibrinous deposit (1, 2). The function of fibrin in the body is to restore injured tissues. Fibrin is a sticky substance and the exudate formed establishes a bridge between the damaged surfaces (3). This process usually starts within 3 hours following injury (4). Formation of adhesion begins during the inflammatory stage of healing, 24 to 48 hours after injury, and the adhesions usually are well formed by 5 to 7 days after injury (5). Adhesions are thought to cause pain indirectly by restriction of organ motion, thus stretching and pulling smooth muscles of adjacent viscera or the abdominal wall (6). Postoperative formation of adhesions is a well-known complication to surgery. Post surgical adhesion formation is an everyday problem in clinical (7). Approximately 3% of all surgical admissions are associated with intra-abdominal adhesions (8). Postoperative adhesions, however, account for 40% of all cases of intestinal obstructions, and 60-70% of those involve small bowel (9, 10). For many years currently, there is no ideal method or real evidence for complete effectiveness in preventing postoperative adhesion formation (1, 11 and 12). Multiple adjuvants evaluated to postoperative adhesion prevention. They include agents that prevent inflammation such as; steroidal and non-steroidal anti-inflammatory medications, bioabsorbable membrane, adhesion prevention gels, viscous solution. Recently Suprogel®- macromolecule polysaccharide evaluated for reduction of postoperative abdominal adhesions formations in rats (13). Glycerol is one of the most common alcohols found in metabolism. It is a 3-carbon molecule containing three hydroxyl groups. Glycerol is the backbone of triacylglycerols (triglycerides or natural fats) and phospholipidis (14). Glycerol (synonym: glycerine, glycol alcohol) is a viscous liquid alcohol with a molecular weight of 92.09 daltons. It dissolves in water and alcohols, but not in liquid hydrocarbons (15). Glycerol is used in medical, pharmaceutical and personal care preparations, mainly as a means of improving smoothness, providing lubrication and as humectants (a hygroscopic substance). It is found in cough syrup, elixir and expectorant, toothpaste, mouthwash, skin care products, shaving cream, hair care products and soap (16, 17). To our knowledge, few studies assessed the efficacy of glycerol in preventing of postoperative peritoneal adhesion (PPA). The aim of the study was to evaluate the effect of (14.3% and 28.6%) glycerol on prevention of experimentally induced intra-abdominal adhesions in rat model.

Material and Methods

- **Preparation of sterile glycerol solution 14.3% and 95% concentrations:** Glycerol 14.3% solution was prepared by adding 71.5ml (W/V) from the glycerol 95% (glycerol, fisher Scientific, U.K) to a small amount of sterile physiologic saline solution (NaCl 0.9%) in a sterile graduated glass cylinder. The fluid was well agitated and the volume was completed up to 500 ml with a sterile saline solution (18). Glycerol 28.6% solution was prepared by adding 143ml (W/V) from the glycerol 95% (glycerol, fisher Scientific, U.K) to a small amount of sterile physiologic saline solution (NaCl 0.9%) in a sterile graduated glass cylinder. The fluid was well agitated and the volume was completed up to 500 ml with a sterile saline solution (18).
- **Animals and Experimental design:** The experiments were carried out on 30 male wistar albino rats weighing between 200-250 g. Animals were housed at 21c° with a day/night cycle of 12hours. They had free access to water and standard rodent feed. Rats were divided randomly into three groups. Treatment 1 and 2 (T1 and T2) each contains 12 rats, and the control group (C) contains 6 rats.
- **Anesthesia and Surgery:** The rat's weight in gram was determined individually before administration of anesthetic drugs that was induced with a mixture of ketamine hydrochloride (75mg/kg) (Holden-Medical, Netherlands, 50mg/kg) and xylazine hydrochloride (3mg/kg) (CEVA GmbH, 10mg/kg), by intraperitoneal injection (19). Rats were positioned on dorsal recumbence and prepared for aseptic abdominal surgery. The abdomens were shaved and prepared with 10% antiseptic poviden-iodine solution. Ventral midline celiotomy, 2-3 cm in length create and the lower caudal ileum and cecum were exteriorized. The antimesentric border of the proximal end of the cecum was used for cecotomy. In each rats, the caecotomie approximately 2 cm length performed. The cecotomies were closed in one layer: a simple interrupted pattern (4-0 chromic gut). The cecum was subsequently returned to the abdominal cavity. Prior to last stitch abdominal closure solutionsinjected to peritoneal cavity depending on the groups:

T1: 5ml of sterile glycerol 14.3% was injected.
T2: 5ml of sterile glycerol 28.6% was injected.
C: 5ml of sterile normal saline was injected.

The linea alba and subcutaneous tissue were closed with 3-0 chromic gut in a simple continuous pattern. The skin was closed with 2-0 silk interrupted pattern. The animals were resumed their normal diet from 1st post- operative day till 14th post- operative day.

- **Assessment of Adhesions**: Two weeks later, all rats were scarified after administration of lethal dose of ketamine given intraperitoneally. The abdominal cavities were opened using wide inverted "U" incisions that extended from the lower margin of the thorax to the inguinal ligaments. The abdominal walls were slowly retracted inferiorly under observation with a surgical retractor to prevent avulsing adhesions and for complete intraperitoneal exploration. The adhesions were graded in a blinded fashion utilizing the classification system described by (20) Table (1).

Table (1) Macroscopic postoperative adhesions grading

Grades	Descriptions
0	Complete absence of adhesions
1	Single band of adhesion between viscera or from one viscus to the abdominal wall
2	Two bands, either between viscera or from viscera to the abdominal wall
3	More than two bands between viscera or from viscera to the abdominal wall
4	Multiple dense adhesions, or viscera directly adherent to the abdominal wall and extent of adhesive bands

- **Histopathology:** Adhesion-carrying tissues to be excised en-block and fixed in a 10% neutralbuffered formaldehyde solution. Samples submitted for histological analysis after being stained with hematoxylin and eosin dye. The parameters to be evaluated were; fibrosis, inflammation, and vascular proliferation, rated on a modified semi-quantitative scale of 0-3 (21). Table (2).

Table (2) Microscopic histopathologic adhesions parameters

Adhesions Parameters	Grade 0	Grade 1	Grade 2	Grade 3
Fibrosis Score	no fibrosis	minimal, loose fibrosis	moderate fibrosis	florid dense fibrosis
Inflammation Score	no inflammation	presence of giant cells, occasional lymphocytes and plasma cells	presence of giant cells, plasma cells, eosinophils and neutrophils	presence of many inflammatory cells and microabscses
Vascular Proliferation	no vascular proliferation	mild vascular proliferation	moderate vascular proliferation	intense vascular proliferation.

- **Statistical Analysis:** Duncan's multiple range tests (22) were used to compare between the means of microscopic adhesion scores. A value of $p \leq 0.01$ considered statistically significant.

Results

All animals recovered from anesthesia with no evidence of complications. The distribution of adhesions with different grades in the groups (T1, T2, and C) shown in Table (3). (T1) 6 rats out of 12 had grade 1 adhesion and remains 6 rats had grade 3 adhesion. (T2) 4 rats out of 12 had grade 1 adhesion, 6 rats from 12 had grade 3 and remains 2 rats had grade 4 adhesion. All control rats had graded 4 adhesions. In the experimental rats, the majority of fibrous adhesion bands were developed between the cecotomy site-visceral organs (Fig.1, 2 and 3). The microscopic adhesion scores and their statistically analysis were shown in Table (4). Microscopic fibrosis scores were significantly different among T2 and C group animals ($p \leq 0.01$), while the differences between T1 and C animals were not significant ($p \geq 0.01$). The means of microscopic inflammation scores statistically not differ significantly among the three groups. The means of microscopic vascular proliferation scores was significantly ($p \leq 0.01$) higher in C animals comparing with T1 and T2, whereas the differences between T1 and T2 were not significant. Histopathological sections obtained are illustrated in(Fig.4,5and 6).

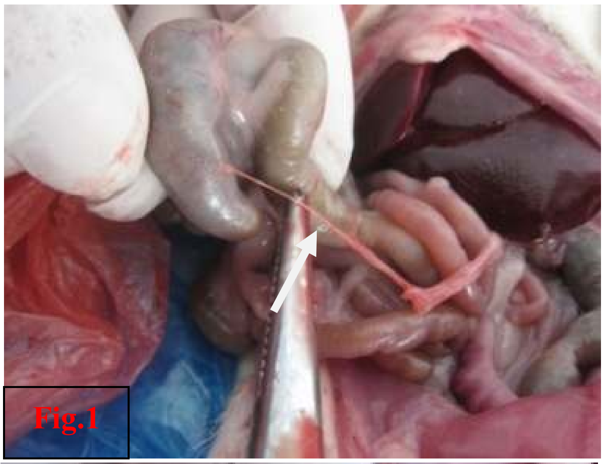
Table (3) Disturbance of rats with different grades of adhesions in the three groups

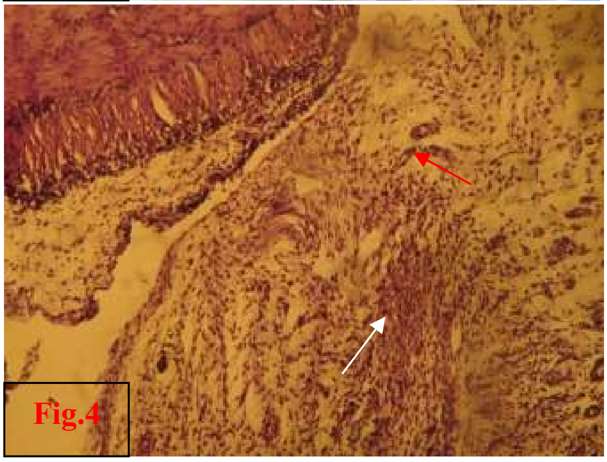
Grade	T1 n= 12	T2 n=12	Control n =6
0	0	0	0
1	6	4	0
2	0	0	0
3	6	6	0
4	0	2	6

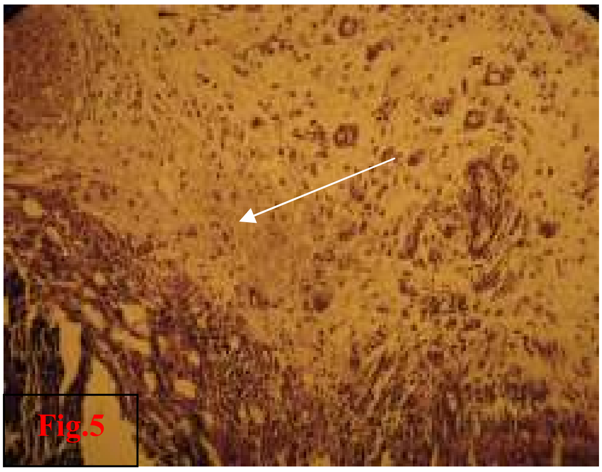
Table (4) Microscopic adhesion scores of the groups and their statistically analysis with Duncan's multiple range test

Groups	No. Animals	Fibrosis Score	Inflammation Score	Vascular Score
T1	12	$1.16 \pm 0.40_{ab}$	$1.66 \pm 0.33_{a}$	$0.66 \pm 0.21_{b}$
T2	12	$0.83 \pm 0.30_{b}$	$1.33 \pm 0.21_{a}$	$0.83 \pm 0.30_{b}$
Control	6	$2.33 \pm 0.33_{a}$	$2.0 \pm 0.0_{a}$	$2.0 \pm 0.0_{a}$

Means within each column with the same letter are not significantly different.







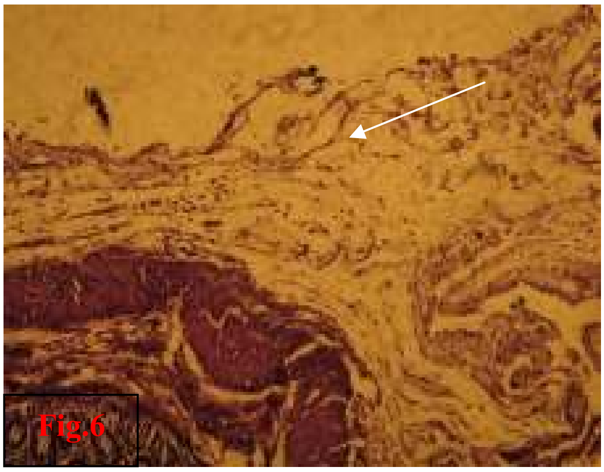


Fig. (1) Grade 1 adhesion between abdominal viscera in a rat from T1.
Fig. (2) Grade3 adhesion between abdominal viscera in a rat from T2.
Fig. (3) Grade 4 adhesion between abdominal viscera in a rat from C.
Fig. (4) Histopathological sections from group C show florid denes fibrosis, infiltration of inflammatory cells (giant cell, plasma cell, eosinophils, neutrophil) (white arrow) and intense vascular proliferation (red arrow). H & E 10X.
Fig. (5) Histopathological section from (T1) group show minimal, loose fibrosis, infiltration of inflammatory cells (giant cell, lymphocytes and plasma cell) (arrow) and mild vascular proliferation. H & E 10X.
Fig. (6) Histopathological section from (T 2) group show minimal, loose fibrosis, infiltration of inflammatory cells (giant cell, lymphocytes and plasma cell) (arrow) and mild vascular proliferation. H & E 10X.

Discussion

Movements between organs and the abdominal wall are necessary to accommodate peristalsis and the highly varying volume of the intestines. Post-operative adhesions can limit these movements, causing pain and other pathology. In this study, in a rat model, we hypothesized reduction in post-operative adhesions formation by injecting two different concentrations of sterile glycerol 14.3% and 28.6%.Adhesions are major complications in healing following surgery or infection and can lead to conditions such as intestinal obstruction, infertility, and chronic pain (23 and 24). They formed when the parietal or visceral peritoneum is damages and the basal membrane of the mesothelial layer is exposed to surrounding tissues (23, 24, 25, and 26). In this study, we used caecotomy as a model in rat for development of adhesion, because caecum appears to be a suitable intra-abdominal organ for the study of surgical adhesions (27). The hypothesis in the present study, behind the suggested purpose for application of glycerol, was to act as a hyperhydrating agent when mixed with physiological normal saline and as a lubricant for reduction of congestion and trauma which usually accompanying serosal abrasions. Recently (28) revealed that covering peritoneal surfaces with 0.1 ml of concentrated glycerol in rat model, both before and after peritoneal trauma, was effective in decreasing peritoneal adhesion formation. The efficacy of glycerol covering was greater in the group receiving glycerol prior to trauma because it decreased the direct effects of trauma on the surfaces. They thought that the reasons for testing of glycerol were: its biocompatibility and its ability to permanently coat all surfaces with which it is in contact, and assurance of its potential non toxic reaction in the peritoneal cavity. In this study, intra-peritoneal application of two different concentrations of glycerol following laparotomy and caecotoy was aimed to assess the role of glycerol in prevention or reduction of postoperative intra-peritoneal adhesions, because glycerol had a hyperhydrating and floating activity, due to its viscosity that asses wound healing causing a layer between surfaces, thereby lubricant action between exposed serosal surfaces. Furthermore, the use of two different viscosities dilutes to prevent and/or to reduce intraperitoneal adhesions that were without any obvious toxic reactions are required to confirm our results. We observed that the potency of grossly adhered parameters in group C was significantly higher than those in T1 and T2groups.This means that the majority of adhesions in the treatments groups had single or more than two bands between viscera to viscera, while the non-glycerol treated rats (C group)showed multiple densebands of adhesions. The histological findings on adhesions showed that the (C group) had the highest fibrosis score and lower fibrosisscores among (T1 and T2) this agrees with (28).(6) Suggested that angiogenesis may play a regulating the growth of nerve fibers into adhesions andcausing pain. This agrees with our results whichvascular proliferation and inflammation scores in (C group) had the highest score among (T1 and T2). Development of a bioabsorbable membrane containing up to 23 percent glycerol and chemically modified sodium hyaluronate/ carboxymethylcellulose offers ease of handling and is shown to provide significant postoperative adhesion prevention in animals (29). From all above, the present rat- model studyindicated that following laparotomy itis beneficial toinject glycerol into the abdominal cavity, as it had a role in reduction of postoperative adhesions. This finding agrees with others (28, 29, and 30). In conclusion, direct instillation of intra-peritoneal glycerol (14.3%, 28.6%) in rats significantly reduced intra-abdominal adhesion formation after caecotomy.

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