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Original article

Comparison of honey and dextrose solution on post-operative peritoneal adhesion in rat model

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ABSTRACT

Background: Peritoneal adhesion between abdominal organs is a complication of surgery. It causes major complications like pain, bowel obstruction, infertility and increases risk of death. Honey is known to have anti-inflammatory and antioxidant properties potentially relevant for adhesive protection.
Methods: Thirty rats were divided into five groups: negative control without any surgical procedure (normal group), control group treated with normal saline, experimental group I treated with 1 ml of 10% honey, experimental group II treated with honey at half concentration of group I (honey0.5), and positive control group receiving 1 ml of dextrose 5%. Inflammatory, growth and angiogenesis factors (TNF-α, Il-6, IL-1β, TGF-β1 and VEGF) of the adhesion tissue were assessed using ELISA. Antioxidant factors (NO, GSH and MDA) were also assessed using biochemical procedures.
Results: The difference between peritoneal adhesion scores, TNF-α, IL-1β, IL-6, TGF-β1, VEGF, NO, GSH and MDA value of all groups was strongly significant ($p < 0.001$). We showed that honey can decrease peritoneal adhesion ($p < 0.001$), TNF-α ($p < 0.001$), IL-1β ($p < 0.001$), IL-6 ($p < 0.001$), TGF-β1 ($p < 0.001$), VEGF ($p < 0.001$), NO ($p < 0.001$), MDA ($p < 0.001$) and increase GSH ($p < 0.001$) compared with control group. Honey 0.5 also significantly decreased peritoneal adhesion ($p < 0.001$), TNF-α ($p < 0.001$), IL-1β ($p < 0.01$), IL-6 ($p < 0.001$), VEGF ($p < 0.001$), NO ($p < 0.001$), MDA ($p < 0.01$) and increase GSH ($p < 0.001$) compared with control group.
Conclusions: We find that honey can decrease inflammatory, growth and angiogenesis factors which can advance peritoneal adhesion and increase antioxidant factors. Honey could serve as a protective agent for peritoneal adhesion.

1. Introduction

Peritoneal adhesions are linkages created between omentum, loops of intestine and abdominal walls. These linkages are made up of connective tissue, blood vessels and nerve tissue, and may be formed due to direct adhesions between organs surfaces [1]. The most important reasons for peritoneal adhesion are pelvic and abdominal surgeries which cause adhesions at operative sites, non-operative sites or after the lysis of previous adhesion [2–4]. Peritoneal adhesion can potentially cause major complications like pain, bowel obstruction and infertility [5,6]. About one-third of open abdominal or pelvic surgeries will have direct or indirect adhesive complications with an average frequency of two times in the subsequent ten years and a prevalence of 20% within the first year [3,7–9]. Post-operative peritoneal adhesion increases mortality and morbidity, in addition to imposing considerable financial burden. Pathophysiology of peritoneal adhesion is the result of an interplay among acute inflammatory responses [10], fibrinolysis [11,12], and activity of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) [13]. Apart from trauma, infection and tissue ischemia, peritoneal injury causes inflammatory reactions and proliferative responses of injured mesothelial cells [14]. Damaged mesothelial cells and evoked inflammatory cells release cytokines such as tumor necrosis factor(TNF)-α, interleukin (IL)-1β and interleukin (IL)-6, and growth factors such as tumor growth factor-β (TGF-β), insulin growth factor-1 (IGF-1) and platelet-derived growth factor (PDGF) which call acute phase cells

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and growth stimulation of mesothelial cells. Proinflammatory cytokines are important in adhesion stimulation because of their role in fibrinolytic pathways and contribution to the remodeling of extracellular matrix [15]. Rats treated with a combination of antibodies against IL-1 and TNF-α have significant adhesion reductions compared with control group [16]. Serum levels of IL-1 and TNF-α have also been shown to serve as reliable markers for postoperative peritoneal adhesion in humans [10].

Mesothelial cells and macrophages also produce tissue factor (TF) which triggers coagulation cascade and formation of fibrinous matrix [17]. There is a balance between deposition and degradation of fibrin in normal peritoneal cavity which is almost preserved by tissue-type plasminogen activator (tPA) and plasminogen activator inhibitors (PAIs) [18]. Impaired balance between deposition and degradation of fibrin is another major reason for peritoneal adhesion. Complete degradation of fibrin of abdominal injury site causes normal peritoneal healing while incomplete degradation of fibrin creates a place for growing of fibroblasts and growth of new blood capillaries mediated by angiogenic factors to form peritoneal adhesions [19]. Fibroblasts organize to collagen-secreting configuration which leads to fibrosis adhesion formation [20,21].In addition to cytokines, plasmin, PAs and PAIs, there are some other important factors like TGF-β and Matrix metalloproteinases (MMPs). TGF-β secreted by macrophages and platelets has been demonstrated to enhance expression of PAI-1 [22] and dose-dependently reduce fibrinolytic capacity in omentum [23] and peritoneal mesothelial cells [24]. There is strong support that overexpression of TGF-β is associated with adhesion formation. MMP and their inhibitors (TIMP) secreted by mesothelial cells regulate degradation of injured ECM and have an important role in wound healing. However, this equilibrium is altered in injury condition and is another important factor to form peritoneal adhesion [13]. So far a lot of various local and systemic materials have been examined to control intra-abdominal adhesions like anticoagulants, fibrinolytics, anti-inflammatories, antihistamines, growth factor inhibitors and modulators, MMPs, immunosuppressives, locally and systemically administered antibiotics, phospholipids, collagen inhibitors, hypoestrogenic environment inducers, etc. [25] and some have been FDA approved [26,27]. Although there are many material and methods created to solve the peritoneal adhesion problem but they can't still overcome.

Honey is a valuable production of bees which is used from thousands of years in various wound healing [28,29]. Exact mechanism of honey to reduce the wound healing isn't clear but what can be said is that honey due to possessing of various beneficial anti-inflammatory and antioxidant compounds including flavonoids-apigenin, kaempferol, pinocembrin, quercetin, galangin and phenolic acid compounds, caffeic, ellagic, ferulicacids,ascorbic acid, catalase, inhibin, superoxide dismutase (SOD), reduced glutathione, amino acids and selenium.Its role as a versatile material and comes through different routes for wound healing [30–34].

The aim of this comprehensive study is assessing the anti-inflammatory, antioxidant effect of honey and comparison of its effects with same concentration of dextrose solution on peritoneal adhesion.

2. Materials and methods

2.1. Drugs and chemicals

Ethanol was purchased from Sigma®, USA.IL-1β, TNF-α, IL-6, TGF-β and VEGF rat ELISA kits were obtained from Bender Med® company, Germany. Ketamine and Xylazin were from ChemiDaru®, Iran. Other chemicals were from Sigma, USA. The mouse NF-κB p65 (total) ELISA kits was provided from Abcam, USA.

2.2. Animals

Wistar-Albino male rats weighting 275 ± 25 g with age of 8 weeks were used in this experiment. Rats were acclimatized and housed in separated standard cages, in ventilated room with 12 h of light following 12 h of darkness cycle and 60 ± 3% humidity at a temperature of 21 ± 2 °C. Also, food was quite at their disposal before and after experiments.

2.3. Honey collection and preparation of solutions

Honey was purchased from local markets of Mashhad, Khorasan Razavi, Iran. To prepare honey solution, 10 g of honey was dissolved in 100 ml of sterilized distilled water (10% w/v) and shacked well to have clear solution.

2.4. Surgical procedure

The methods were used in our experiment confirmed by ethical committee based on the guidelines of animal experiments in Mashhad University of Medical Sciences. General anesthesia was achieved using an intraperitoneal (i.p.) injection of ketamine (100 mg/kg) and xylazin (10 mg/kg). Subsequently, abdominal region was shaved, cleaned and prepared for surgery with alcohol and iodine solution. A 3 cm laparotomy was carried out to access to abdominal cavity. For inducing adhesion, 4 button-like protrusions (1 cm^2) were generated with an interval of 0.2 cm by 6-0 polypropylene absorbable yarn sutured in cecum peritoneal cavity. After intervention, the cecum was returned to the abdomen and abdomen wall closed with 4-0 polygelatin suture. The procedures were kept to minimum and up to 10 min.

2.5. The studied groups

Thirty male wistar rats were randomly divided to 5 groups of six:

1: Negative control without any surgical procedure: (Normal group),

2: Control group with normal saline treatment,

3 and 4: Experimental group with 1 ml of 10% (honey) and 5%w/v (honey 0.5) of honey treatment (Honey)

5: Experimental group with 5% (w/v) concentration of dextrose solution

All treatments were sprayed in surgical zone with 1 ml syringe. After surgery, rats were kept their cages in recovery room for seven days. At the seventh day of surgery, rats were underwent laparotomy and post-operative peritoneal adhesion scored with a group of observers who were blinded about the study groups and performed using adhesion score published by Nair et al. [35]. Also, cecum and peritoneal lavage fluid was collected for evaluation of inflammatory cytokines and oxidative biomarkers.

2.6. Assessment of inflammatory biomarkers

The peritoneal fluid species were analyzed in accordance with manufacturer's Instructions the relevant ELISA kits. IL-1β, TNF-α and IL-6 rat concentrations, *indexes of inflammation*, were measured.

2.7. Evaluation of fibrosis and angiogenesis biomarkers

The level of TGF-β (*as fibrosis biomarker*) and VEGF (*as angiogenesis biomarker*) were measured in peritoneal fluid species