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Evaluation of the effects of Iranian propolis on the severity of post operational-induced peritoneal adhesion in rats

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ABSTRACT

Background: Peritoneal adhesion is a major complication of surgery that can lead to serious problems such as bowel obstruction, pain, infertility and even mortality. Propolis is a honey bee product with anti-inflammatory and anti-oxidant activities that could potentially protect against adhesive surgical complications.

Methods: Forty 8-weeks-old rats (275 ± 25 g) were divided into five groups: normal group without any surgical procedure, and experimental groups treated with normal saline, 50 mg/kg, 100 mg/kg and 200 mg/kg of propolis. Peritoneal adhesions were examined macroscopically and also, the levels of inflammatory factors (IL-6, IL-1β and TNF-α), growth factors (TGF-β1 and VEGF) were evaluated in the study groups using ELISA. Biochemical indices of oxidative status including Nitric Oxide (NO), Malondialdehyde (MDA) and Glutathione (GSH) were also measured.

Results: Peritoneal adhesion scores, IL-1β, IL-6, TNF-α, TGF-β1, VEGF, NO, GSH and MDA levels were significantly different between the study groups ($p < 0.001$). Propolis treatment reduced 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 ($p < 0.001$) and MDA ($p < 0.001$), while GSH levels were increased ($p < 0.001$) compared with the vehicle group. Our results showed that higher dose of propolis was associated with significantly greater reductions in peritoneal adhesion ($p < 0.001$), TNF-α ($p < 0.001$), IL-1β ($p < 0.001$), IL-6 ($p < 0.001$), VEGF ($p < 0.001$), NO ($p < 0.001$) and MDA ($p < 0.001$), a greater increase in GSH levels ($p < 0.001$) compared with the lower dose.

Conclusions: Propolis treatment can dose-dependently reduce peritoneal adhesion through its anti-inflammatory, anti-angiogenic and antioxidant properties. Therefore, propolis might serve as a protective agent against post-surgical adhesive complications.

1. Introduction

Peritoneal adhesion is known as a major complication of abdominal surgery that occurs in 93–100% of subjects undergoing surgery [1,2]. The complication is comprised adhesions and providing connective tissue between omentum, intestine and abdominal walls as well as made up of blood vessels and nervous tissue [3]. The most common sequel of peritoneal adhesion are chronic pelvic pain, small-bowel obstruction, fistula formation, infarction and female infertility [4,5].

Since wound healing process has a key role in repairing peritonal injury induced by surgical trauma, any disruption in this process such as mesothelial damage and microbial infection can lead to abdominal adhesion [6]. The pathophysiology of adhesion involves aberrant interaction between different biological responses like inflammatory responses, activation of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), imbalance in fibrinolysis, and secretion of various cytokines and mediators [7]. It has been demonstrated that fibroblasts in the adhesion site have higher mRNA levels of MMPs, TIMPs, collagen type I, fibronectin, pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, tumor growth factor-β (TGF-β), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). These factors have a key role in

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adhesion stimulation because they contribute to fibrinolytic pathway, proliferation of endothelial cells, development of vascular structure and remodeling of extracellular matrix in the adhesion site [8]. Hypoxia also promotes the production of inflammatory cytokines such as IL-6 and TNF-α, and contributes to the development of postoperative adhesions [9]. Hitherto, various strategies have been tried to reduce abdominal adhesions such as optimizing surgical procedures, improving laparoscopic techniques, using different pharmacological agents which interfere with the adhesion process or fibrin deposition (such as anti-inflammatory agents, anticoagulants, growth factor inhibitors, fibrinolytics, collagen inhibitors, thromboxane A2 receptor blockers and immune-suppressives); yet limited success has been attained [10]. Therefore further studies are needed to find more effective agents that could be safely used or the prevention and treatment of post-surgical peritoneal adhesion.

Propolis is a natural product produced by honeybees from several plants [11]. Several studies have demonstrated different biological and pharmacological effects of propolis including antioxidant, anti-inflammatory, immunomodulatory, antiviral, antimicrobial, antifungal and anti-tumr properties [12].

In the last decade, some anti-inflammatory compounds of prospolis have been identified including naringenin, caffeic acid, caffeic acid phenethyl ester (CAPE), quercetin, salicylic acid, ferulic acid, galangin and apigenin [13]. Of note, anti-inflammatory effect of propolis has been reported in improving wound healing [14,15]. Several reports have shown that propolis ingredients can inhibit NO release from macrophages [16]. Also, CAPE has been shown to promote the activities of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) [17]. Several studies have demonstrated that CAPE inhibits the transcription factor NF-κB and different downstream pro-inflammatory cytokines such as TNF-α, IL-6 and IL-13 [18,19]. Moreover, flavonoids of propolis can eliminate free radicals which can potentially contribute to the regeneration and healing of damaged tissues [11].

Owing to the above-mentioned beneficial effects of propolis, this study aimed to evaluate the effectiveness of this natural product in preventing peritoneal adhesion. We also explored the effects of propolis treatment on inflammation and oxidative stress as two major pathological mechanisms involved inpost-surgical peritoneal adhesion.

2. Materials and methods

2.1. Drugs and chemicals

IL-6, IL-1β, TGF-β, TNF-α, and VEGF rat ELISA kits were prepared from Bender Med® company, Germany. Ethanol was purchased from Sigma®, USA. NO, MDA and GSH biochemistry kits were purchased from ZellBio Company, Germany. Ketamine and Xylazin were from ChemiDaru®, Iran. Other chemicals were purchased from Sigma, USA. The injectable normal saline serum (normal saline) was also prepared from local pharmacy store which produced by Samen® pharmacy factory, Iran.

2.2. Animals

A total of 40 healthy 8-weeks-old male Wistar-Albino rats weighting of 275 ± 25 g were purchased from the animal holding unit, department of Medical Sciences in Mashhad University (Mashhad, Iran). Rats were acclimatized and housed in separated standard cages. The animal room was well ventilated with temperature of 21 ± 2 °C and 60 ± 3% humidity with 12 h natural light-dark cycle. Rats were allowed free access to standard tap water and laboratory pellet chow. Good hygiene was maintained by constant cleaning and removal of feces and spilled feeds from cages daily. All animals received humane care in compliance with institutional guidelines.

2.3. Propolis collection and preparation of solutions

Propolis samples were collected from bee keeping areas of Mashhad, Khorasan Razavi, Iran. Twenty grams of propolis was immersed in 500 mL of 70% hydro-ethanolic solution and incubated for 48h along with shaking. Subsequently, the solution was concentrated using rotary evaporator and free dryer, resulting in dry extract [20,21]. To prepare the solution, 1000, 500 and 250 mg of the extract were dissolved in 5 mL of normal saline and administrated 0.1 mL/100 g body weight of mice for each 200 mg/kg, 100 mg/kg and 50 mg/kg doses treatment of propolis, respectively. The solutions were also prepared freshly and daily.

2.4. Assessment of total phenolic contents (TPC) of the extract

TPC of extract was defined using Folin–Ciocalteu (FC) reagent which described previously [22,23] with minor modification as followed. Briefly, Fraction (100 µl) of ethanolic solution of propolis (20 µg/mL) was mixed equal volume of water in test tube. Next, about 200 µL of FC reagent were augmented to tube. Following next 2 min, 2600 µL of a 5% (w/v) sodium carbonate solution were added. The mixture was incubated at 40 °C for 20 min along with fine shaking. The tubes were then quickly were made cool and the developed color was read at 760 nm using a MultiSpec UV–vis spectrophotometer (Shimadzu, Tokyo, Japan). Estimation of phenolic compounds was carried out regarding to polyphenol reference calibration curve of ethanolic solution of Gallic acid (GA) in range of 0.5–10 mg/L [23]. The amount of TPC was expressed as mg of GA equivalent (GAE) per gram of dry extract. For blank, same process was performed with 100 µl of distilled water instead of extract.

2.5. Ferric reducing ability of plasma (FRAP) assay

The test was done as stated method Benzie and Strain with some modifications [24]. In brief, the FRAP reagent was made using following mixture:

- 2.5 mL of a solution of 2, 4, 6-tripyridyl-s-triazine (10 mM) in 40 mM HCl, 2.5 mL of $FeCl_3$ (20 mM) and 25 mL of acetate buffer (0.30 M, pH = 3.6).

Sample (100 µL) were mixed with 300 µL of distilled water and 3 mL of FRAP reagent to provide a final concentration of 20 µg/mL of the extract. The absorbance of the mixture was read using ELISA reader, at 595 nm. The standard calibration curve was prepared with Trolox® (0.02–10.50 mg/L). The finding was pronounced as Trolox equivalent antioxidant capacities (TEAC), as mg of Trolox per gram of dry extract.

2.6. Surgical technique

All procedures involving animals were approved by ethical committee based on the guidelines of animal experiments in Mashhad University of Medical Sciences. For anesthesia the animal received 100 mg/kg of ketamine and 10 mg/kg xylazin intraperitonealy. Surgical procedure was performed according as describe previously [25]. Briefly, after shaving and disinfection of the skin with alcohol and iodine solution, a 3 cm incision was made carefully to access abdominal cavity. In order to induction of adhesion, on one side of the cecum peritoneal cavity, 4 button-like protrusions (1 cm^2) with an interval of 0.2 cm with sterilized and absorbable 6–0 yarn were generated. After intervention, the abdominal incision was closed with 4–0 polygelatin suture. After surgery, rats were kept in their cages in recovery room for fourteen days. Prolonged time to surgery was kept to minimum and at least 10 min.