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Administration of honey to prevent peritoneal adhesions in a rat peritonitis model

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ABSTRACT

Aim: We investigated the effects of intraperitoneal honey on the development of postoperative intra-abdominal adhesions and oxidative stress in a model of bacterial peritonitis.

Methods: Bacterial peritonitis was induced in 18 rats by cecal ligation and puncture. The rats were randomly assigned to three groups. Group 1 ($n = 6$) received honey intraperitoneally, group 2 ($n = 6$) received 5% dextrose intraperitoneally, and the third group received no fluid or medicine intraperitoneally one day after cecal ligation and puncture procedure. All animals were killed 14 days later so we could assess the adhesion score. Tissue antioxidant levels were measured in 1-g tissue samples taken from the abdominal wall.

Results: Adhesion scores of honey treated group were significantly lower according to the control group ($P < 0.05$) and statistically significant. Adhesion scores of honey were lower from 5% dextrose but not statistically significant ($P > 0.05$). Malondialdehyde values of honey group were significantly lower from the control group ($P < 0.05$) and levels in 5% dextrose group was higher than the honey group. Catalase levels were high in control and 5% dextrose groups. Superoxide dismutase levels were higher in the control group than the honey group (statistically significant).

Conclusions: Intraperitoneal honey decreased the formation of postoperative intra-abdominal adhesions without compromising wound healing in this bacterial peritonitis rat model. Honey also decreased the oxidative stress during peritonitis.

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1. Introduction

Peritoneal inflammation is one of the main cause of post-operative abdominal adhesions represent intestinal obstruction, infertility and abdominal pain in surgical patients.[1] The inflammatory response has been recognized as a common cause in all pathways for adhesion formation. In recent years, numerous research has shown that leukocyte-dependent inflammatory reactions may increase cellular and tissue injury.[2] Adhesions are the results of the inflammatory response to tissue injury in the peritoneal space.[3] Honey has been used in wound healing and burns since ancient times and in recent years, the accelerative effect of honey in the wound healing process is shown in vivo and in vitro.[4] Honey inhibits the growth of both gram-positive and gram-negative bacteria.[5]

In this study, we investigated the effects of intraperitoneal honey on the development of postoperative intra-abdominal adhesions and oxidative stress in a model of bacterial peritonitis.

2. Materials and methods

We used 18 female Wistar rats weighing 200–220 g in this study. Animals were housed at 21 °C and given standard rat chow diet and water ad libitum. The study protocol was approved by the Animal Ethics Review Committee of the Faculty of Medicine, University of Kahramanmaras.

2.1. Surgical procedures

Bacterial peritonitis was induced in all rats, by a cecal ligation and puncture (CLP) procedure, using the methods of Wichterman et al.[6,7] All the rats were in the same stage of the estrous cycle. The vaginal smear technique procedure was used for determining the estrous state in rats. This procedure performed by our pathology department. The animals were not given food for 12 h before the first operation. All animals were anesthetized with a mixture of

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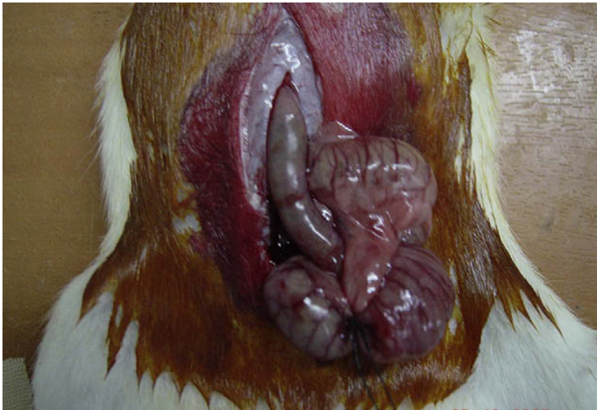

Fig. 1. The caecum was ligated just proximal to the ileocecal valve, with a 3/0 silk suture, and at the anti-mesenterial site.

40 mg/kg ketamine and 5 mg/kg xylazine hydrochloride. The abdomen was shaved and swabbed with a povidone iodine solution preoperatively. The same researcher performed all surgical procedures. A 3 cm midline incision was made and the abdomen was opened under clean surgical conditions. The caecum was dissected without damaging the vascularization and was filled backwards with faeces. Thereafter, the caecum was ligated just proximal to the ileocecal valve, with a 3/0 silk suture, and at the anti-mesenterial site the caecum was punctured twice with a 22-gauge needle, squeezed gently to force out a small amount of faeces, and then returned to the abdominal cavity. Then the midline incision was closed in one layer with a 3/0 silk suture. (Fig. 1) Immediately after the operation, all animals were resuscitated with 5 mL of isotonic sodium chloride solution administered subcutaneously. On day 1, all animals were operated again under anaesthesia, and peritoneal fluid samples were taken for microbiological examination. The cecal ligated stump was isolated and resected. Before closure of the abdomen, the rats were randomly assigned to three groups: Group 1 ($n=6$) received single dose of 1 mL honey intraperitoneally, group 2 ($n=6$) received single dose of 5 mL 5% dextrose intraperitoneally and group 3 ($n=6$) was a control, which did not receive any injection. (Fig. 2) All animals were given water from the first postoperative day; standard rat chow and water were provided on the second postoperative day. Samples of peritoneal fluid were cultured in aerobic and anaerobic conditions.

In this study, the honey used was of pine tree origin, manufactured from south part of Turkey trees.

For aerobic culture, the samples were inoculated onto 5% sheep blood agar, chocolate agar and Mac Conkey's agar and incubated for 24–48 h at 35 °C. For anaerobic culture, samples were inoculated onto chocolate agar, Mac Conkey's agar, 5% anaerobic sheep blood agar containing canamycin and vancomycin, and incubated for 48–72 h at 35 °C in a GasPak anaerobic system. In addition, the samples obtained by swabbing for the anaerobes were taken into the enriched thioglycolate broth, and incubated for 4–7 days at 35 °C in a GasPak anaerobic jar (AnaeroGen, OXOID, Basingstoke, England). Aerobic microorganisms were identified by standard laboratory methods and API ID32E. Anaerobic microorganisms were identified by using OXOID Anident discs.

All rats were sacrificed on the 14th day after being anaesthetized with overdose ethyl ether before relaparotomy. The abdomen was opened with an inverted U incision. One point was given for each adhesion and a cumulative adhesion score was calculated. Adhesions were scored in a blinded manner according to the method of Bothin et al. (Table 1).[8]

In order to determine tissue antioxidant levels, 1×1 cm^2. Tissue samples were taken from the lateral of the incision line on the left abdominal wall. The samples were preserved in a deep freezer until examination. The tissues were homogenized with three volumes of ice-cold 1.15% KCl. The activities of antioxidant enzymes and the levels of lipid peroxidation were measured in the supernatant obtained from centrifugation at 14,000 rpm. Superoxide dismutase (SOD) activity was measured according to the method described by Fridovich.[9] Catalase (CAT) activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Beutler.[10] Lipid peroxidation level in the tissue samples was expressed in malondialdehyde (MDA) and measured according to the procedure of Ohkawa et al.[11] Protein concentration was determined according to the method of Lowry et al.[12]

2.2. Statistical analysis

All variables were expressed as mean and standard error. Differences between groups were evaluated by Kruskal–Wallis variance analysis followed by a post hoc Mann–Whitney *U*-test. *P*-values <0.05 were considered statistically significant. All data were entered into and processed by SPSS 9.05 for Windows statistical package.

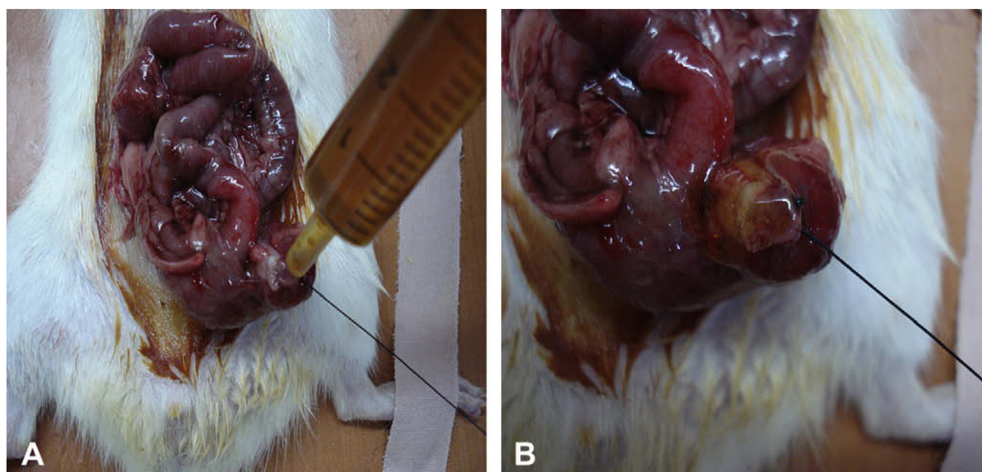

Fig. 2. Administration of honey to caecum stump, intraperitoneally. A: Resected caecum postoperative 2nd day and 1 mL honey administration with 5 mL syringe. B: Administered honey to resected caecum.

Table 1
Cumulative adhesion scoring scale.[7]

Points	
0	No adhesions
+1	One adhesion band from the omentum to the target organ
+1	One adhesion band from the omentum to the abdominal scar
+1	One adhesion band from the omentum to another place
+1	One adhesion band from the adnexa/epididymal fat bodies to the target organ
+1	One adhesion band from the adnexa/epididymal fat bodies to the abdominal scar
+1	One adhesion band from the adnexa/epididymal fat bodies to another place
+1	Any adhesive band other than described above
+1	Target organ adherent to the abdominal wall
+1	Target organ adherent to the abdominal scar
+1	Target organ adherent to the bowel
+1	Target organ adherent to the liver or the spleen
+1	Any other organ adherent

*Target organ was resected caecum.

3. Results

The results of the adhesion scores in all groups are presented in Fig. 3. The mean ± SE scores on postoperative day 14 were as follows: Group 1 (control), 8.4 ± 0583; Group 2 (honey), 4 ± 0948; Group 3 (5% dextrose), 6 ± 0836. The differences in adhesion scores among the three groups were significant ($P < 0.05$, Kruskal–Wallis test). Adhesion scores of honey treated group was significantly lower according to the control group ($P < 0.05$, post hoc Mann–Whitney *U*-test) and statistically significant. Adhesion scores of honey were lower from 5% dextrose but not statistically significant ($P > 0.05$). When the antioxidant levels of the lateral wall of the abdomen were evaluated at day 14, Group 1 (control), 1604 ± 0361; Group 2 (honey), 0336 ± 0044; Group 3 (5% dextrose), 1392 ± 0202. The MDA values of honey group were significantly lower from the control group ($P < 0.05$) and levels in 5% dextrose group were higher than the honey group (Fig. 4). The values of CAT were as follows: Group 1 (control), 11,258 ± 1259; Group 2 (honey), 4984 ± 1674; Group 3 (5% dextrose), 95,460 ± 0627. CAT levels were high in control and 5% dextrose groups, but the differences between honey group were not statistically significant. If the SOD levels were taken into account, the values were as follows: Group 1 (control), 21,992 ± 2885; Group 2 (honey), 8058 ± 1924; Group 3 (5% dextrose), 16,474 ± 1002. The control group was higher than the honey group(statistically significant). It was detected that the results of the 5% dextrose group for SOD were higher compared to honey group, but it did not reach statistical significance when 5% dextrose is compared with honey (Fig. 5).

The culture results of the samples taken on the first postoperative day revealed polymicrobial intra-abdominal infection. The most frequently isolated microorganisms were *Escherichia coli*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Proteus vulgaris*, group D *Streptococcus*, *Enterococcus*, *Staphylococcus aureus*. *Clostridium difficile* and *Bacteroides fragilis* were the most frequently isolated anaerobic agents. Within 1 day after CLP, 1 rat in each group died due to sepsis.

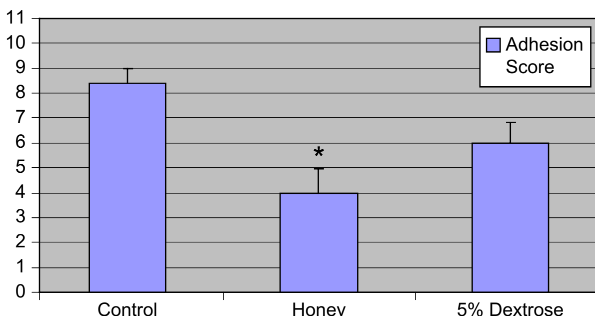

Fig. 3. Adhesion scores. The differences of adhesion scores among all the groups were significant ($P < 0.05$, Kruskal–Wallis test). *The adhesion score of honey group was significantly lower than the control group. ($P < 0.05$, post hoc Mann–Whitney *U*).

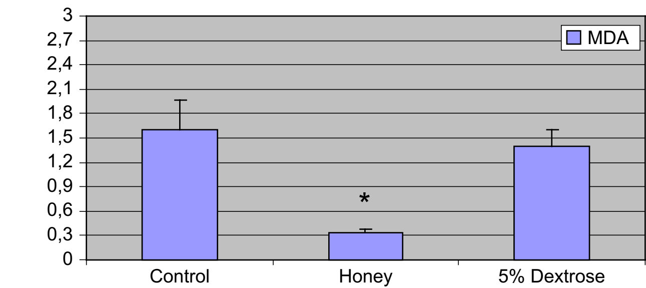

Fig. 4. MDA levels (nmol/mg protein). MDA levels in control and 5% dextrose groups were higher than in the honey group. The differences of MDA levels among all the groups were significant ($P < 0.05$, Kruskal–Wallis test). *The MDA levels honey group were significantly lower than 5% dextrose group. ($P < 0.05$, post hoc Mann–Whitney U).

4. Discussion

Many studies have shown antimicrobial properties of honey and these effects potentiate the wound healing process.[13] Honey inhibits microbial growth because of high osmolarity,[14] but Cooper et al. shows that antimicrobial effect of honey in a different way from osmolarity effect.[15] Honey is composed of several chemically active agents. Also physical properties like hygroscopicity, lower pH, and hypertonicity of honey are supposed to be responsible for its wound healing effect.[5,16,17]

The results of French et al. study clearly showed that honey has the potential to be used as an antibacterial agent to prevent and control infection with coagulase-negative staphylococci application.[18] More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives.[13]

In an experimental study, Bothin et al.[8] showed that the bacterial flora plays a significant role in adhesion formation. We have established our present study on this property that honey may be beneficial as far as infection control and reduced the intra-abdominal adhesions. In our present study, bacterial peritonitis was induced by performing CLP procedure. Significantly lower score of adhesions in the abdominal cavity was found 14 days after instillation of honey compared with 5% dextrose and no fluid or medicine instillation. This findings may support the thesis of a beneficial

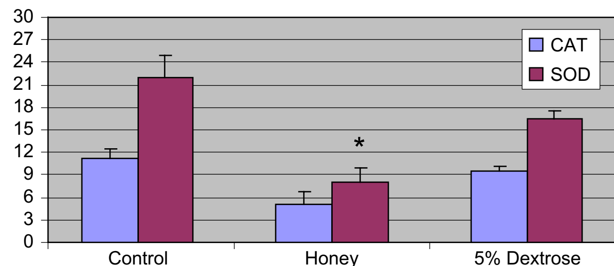

Fig. 5. SOD and CAT levels (U/mg protein). * The SOD levels of honey group was significantly lower than control group. ($P = 0.016$, post hoc Mann–Whitney U). The differences of CAT and SOD levels among all the groups were significant ($P < 0.05$, Kruskal–Wallis test).

effect of honey in bacterial peritonitis. Honey, therefore, would be suitably active for both therapeutic and prophylactic.

Aysan et al showed that administration of honey reduced postoperative peritoneal adhesions in rats significantly.[19] Our study, which showed fewer significant adhesions in the group that administered honey, support these results. Additionally, we found lower MDA values in honey group than control and 5% dextrose groups. This reduction could be attributed to the important role of honey in modulating production of free radicals[20] and antibacterial activity. Honey may also have affected the formation of adhesions through its antioxidant properties, serving as a scavenger of locally generated free radicals. These mechanisms are as yet unknown, but are possibly derived from the phytochemicals present in honey. Furthermore, honey has been shown to produce hydrogen peroxide and dilution of honey is activated catalyses the slow generation of hydrogen peroxide which inhibits bacterial growth.[21] Hydrogen peroxide is a well-known antimicrobial agent, initially hailed for its antibacterial and cleansing properties.[22] The harmful effects of hydrogen peroxide are further reduced because honey inactivates the free radicals.[23]

Several authors are of the opinion that the sugar content of honey is exclusively responsible for its antibacterial effect. For this purpose, we compared effectiveness of honey to that of sugar analogue (5% dextrose). We found MDA levels in 5% dextrose group higher than honey group that shows us the better antioxidant effect of honey from 5% dextrose. This result shows that the effect of honey not only sugar content and osmolarity, but also its additional phytochemical activity.

Another research has shown that honey modulates the activation of monocytic cells in vitro without affecting viability. This modulation gives inhibitory and stimulatory effects. This is important because phagocytic activities are essential for controlling infections and are mediators of inflammation.[24,25] This affect mainly what we require in reducing intraperitoneal adhesion formation. The effects observed in this study may contribute to explain the observed effects of honey on peritoneal adhesion formation with lower adhesion scores and oxidant levels.

We concluded that intraperitoneal honey decreases the incidence of postoperative intra-abdominal adhesion formation without impairing the healing of wound in rats bacterial peritonitis model. Whichever honey is used intraperitoneally, consideration needs to be given to its quality and further evidence and understanding of the therapeutic and chemical properties of honey is needed to optimise the use of this product in the clinical management of postoperative intra-abdominal adhesions.

Conflict of interest

None.

Funding

None.

Ethical approval

The study protocol was approved by the Animal Ethics Review Committee of the Faculty of Medicine, University of Kahramanmaras.

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