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Oral propolis treatment decelerates experimentally induced osteoarthritis in rats.

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ABSTRACT

Propolis has antioxidant, anti-inflammatory and immunomodulatory features. It also has protective effects in human chondrocyte cultures. These effects of propolis are associated to its constituents like pinocembrin, and caffeic acid phenethyl ester. The aim of this study was to expose the effects of propolis in terms of cartilage tissue protection on an experimental osteoarthritis model in rats. Twenty-eight Sprague Dawley rats divided into four equal groups (Arthrotomy: A, Surgical control: SC, Propolis 100: P1, and Propolis 200: P2). Following right knee arthrotomy the medial meniscus was removed in groups SC, P1 and P2. The surgical procedure was concluded after arthrotomy in Group A. A solution prepared from propolis extract was administered from the first day for five weeks orally in doses of 100 mg/kg/day to the rats in the P1 group and 200 mg/kg/day to the rats in the P2 group. At the end of the study, specimens taken from the medial tibial joints were assessed histologically based on the Mankin scoring system. Compared to the SC group the histological results from both of the groups receiving propolis treatment were significantly better ($p<0.001$ for both). However, there was no significant difference between the P1 and P2 groups ($p=0.506$). In conclusion, propolis was observed to reduce cartilage degeneration in an experimental model of OA. We attribute this to the effect to the components that contained in propolis, such as pinocembrin and caffeic acid phenethyl ester.

1. Introduction

Osteoarthritis (OA) is the most common joint disease, which affect more than half of the population aged over 65. It affects load bearing joints such as the hip and knee. It is characterized by progressive joint cartilage degeneration and subchondral bone changes (Bove et al., 2006; Teeple et al., 2013).

Overproduction of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-17 and IL-18) plays an important role in the pathophysiology of OA. These cytokines, especially TNF-α, exacerbate cartilage destruction by increasing the release of matrix metalloproteinases (MMPs), various aggregants and catabolic enzymes. Cytokines such as TNF-α, IL-1, IL-17 and IL-18 also reduce MMP inhibitors and extracellular matrix components which results an imbalance in the anabolic and catabolic process of the cartilage tissue (Bramono et al., 2004; Lorenz and Richter, 2006; Pasternak and Aspenberg, 2009; Ma et al., 2015; Zhang et al., 2015). Therefore, functional and structural loss in joint cartilage become progressive.

The main objectives in the treatment of OA are reducing pain, restoring the joint function and decelerating the progression of the disease (Teeple et al., 2013). There is intensive research on drugs which are altering the course of the disease. Such drugs particularly intend to alter the course of the disease by affecting the inflammatory cytokines that play an important role in the pathophysiology.

Propolis is a natural resin produced by honey bees. It is obtained through collection from various plants, and used as an adhesive (Khalil, 2006). Various studies have referred to its anti-inflammatory, antioxidant, antimicrobial and antitumor properties by reducing the secretion of various MMPs (MMP-1, MMP-3 and MMP-13), prostoglandine E2 (PGE2), nitric oxide (NO), IL-6, IL-1 and TNF-α (Hu et al., 2005;

Khalil, 2006; Gao et al., 2010; Guney et al., 2011; Soromou et al., 2014; Zhang et al., 2015). These effects of propolis are also associated to its constituents like pinocembrin (PB), and caffeic acid phenethyl ester (CAPE) (Borrelli et al., 2002; Cardile et al., 2003; Hu et al., 2005; Yüce et al., 2015; Armutcu et al., 2015; Zhang et al., 2015).

The purpose of this study was to determine the effects of propolis in terms of cartilage tissue protection on an experimental OA model in rats.

2. Experimental Procedure

This study was performed in the Karadeniz Technical University surgical research laboratory following approval from the institutional ethical committee. Twenty-eight Sprague-Dawley rats with a mean weight of 275 gr (250-300 gr) and aged 20 weeks were used. All rats were placed into separate cages. Room temperature was set at 20-24°C and the light: dark cycle was adjusted to a rhythm of 12 hours of light and 12 hours of dark. Standard rat chow and water were provided. Following observation for 1 week, the rats were randomly assigned into one of four groups (Arthrotomy: A, Surgical control: SC, Propolis 100: P1, and Propolis 200: P2).

Surgical procedure

Following a 4-hour fasting, anesthesia was achieved with the intraperitoneally injection of 10 mg/kg xylazine hydrochloride (Rompun: Bayer, Leverkusen, Germany) and 50 mg/kg ketamine hydrochloride (Ketalar: Pfizer, Istanbul, Turkey). Anesthesia was extended when necessary with additional administration of ketamine. The right leg of the rat was shaved and prepared with povidone-iodine solution. Arthrotomy was performed with a medial parapatellar incision to the right knee. In Group A the surgical procedure was concluded after the arthrotomy stage. In order to obtain an experimental OA model in groups SC,P1 and P2 patella was displaced laterally, the medial collateral ligament was severed and the medial meniscus was removed (Bendele, 2001; Janusz et al., 2002). After the operation, all rats were placed into separate cages and permitted unrestricted weight-bearing as soon as they recovered from anesthesia. No antibiotic was used for infection prophylaxis.

Propolis preparation and application

Propolis specimens collected from various regions of Turkey were powdered and mixed. 100 gr propolis was dissolved with distilled water and left to incubate for 24 h. At the end of 24 h, the extract was removed from the stirring incubator and filtered through filter paper. It was then prepared for use by being passed through a 0.22-µm sterile filter. Study solutions of desired concentrations were prepared from stock propolis extract at a concentration of 100mg/ml. One day after the surgical intervention, this solution was administered by oral gavage to the rats in P1 group at a dose of 100 mg/kg/day and to P2 group at a dose of 200 mg/kg/day. This application was repeated every day for 5 weeks. At the end of the 5th week all rats were sacrificed by cervical dislocation.

Histological analysis

The right knee joints were removed. Muscles and soft tissues were removed without damaging the knee joint. The knee joints were fixed in 10% formalin solution for 24 hours then they were kept in 10% formic acid for decalcification. Hydration was performed with ethanol. All specimens were cut in the exact midline in the sagittal plane. The medial sections were then taken and fixed in paraffin blocks in such a way as to remain on the top surface. Serial sections of 5 micrometers in thickness were taken with a microtome in order to obtain the medial tibial joint surface planned for histological analysis. Specimens were taken from the load bearing region of the medial tibial joint. These sections were stained with Safranin-O/FastGreen and examined blindly on the basis of the Mankin scoring system (Pearson et al., 2011) under a light microscope by a histologist (Olympus BX51).

Statistical analysis

The statistical analyses were performed using the IBM SPSS statistics 22. The compliance of the quantitative data with normal distribution was evaluated by Kolmogorov-Smirnov test. Comparison of groups were performed with Mann-Whitney U test. Bonferroni correction was calculated for comparison of the groups and p values less than 0.016 was considered statistically significant.

3. Results

Limping observed on the first postoperative day and gradually improved in the following days. No infection was observed in the surgical wound site. No contracture or patella dislocation was observed in any knee joints taken for evaluation. All rats survived the study period.

The degree of the cartilage tissue injury of the medial tibial joint surface was examined microscopically in terms of cartilage surface integrity, chondrocyte status, amount of matrix staining with Safranin O and the structure of tidemark.

In Group A, the cartilage structure was generally regular, and cells were normal in terms of shape, numbers and morphology. Staining with Safranin O was mildly decreased in three joints and normal in the others. No tissue loss occurred in the joints (Figure 1).

In the SC group, clefts were observed in the cartilage structure toward the middle layer in five rats and toward the calcified layer in two. Hypocellularity was observed in five rats and cloning in two. Staining with Safranin O decreased moderately in six joints and intensely in one joint. Structurally, the tidemark was not crossed in three joints but was crossed in four (Figure 1).

In the P1 group, superficial irregularity in medial tibial cartilage was seen in six joints. Superficial irregularity and a pannus appearance were seen in one joint. In terms of cell structure, cloning was observed in four joints and intense hypercellularity in three. A mild decrease in staining with Safranin O was observed in two joints and a moderate decrease in five. Tidemark integrity was determined in four joints, while vasculature crossed the tidemark in three (Figure 1).

In the P2 group, in terms of cartilage structure, superficial irregularity was observed in three joints. Superficial irregularity and pannus were seen in three and clefts extending to the middle layer in one. In terms of cell structure, cloning was observed in four joints and hypocellularity in three. A mild decrease in Safranin O staining was observed in three joints and a moderate decrease in four. The tidemark was found intact in three joints while vasculature crossed the tidemark in four (Figure 1).

The full histological values obtained from the groups are shown in Table 1. At statistical analysis, all groups differed significantly from Group A ($p<0.001$). In addition, the P1 and P2 groups differed significantly from the SC group ($p<0.001$ for both). However, no significant difference was observed between the P1 and P2 groups ($p=0.506$).

The Mankin scoring values obtained from Group A were almost normal. However, marked worsening was observed in the SC group (total score: 63). The histological scores of P1 and P2 groups decreased markedly when compared to the SC group (total score in the P1 group: 34, total score in the P2 group: 39).

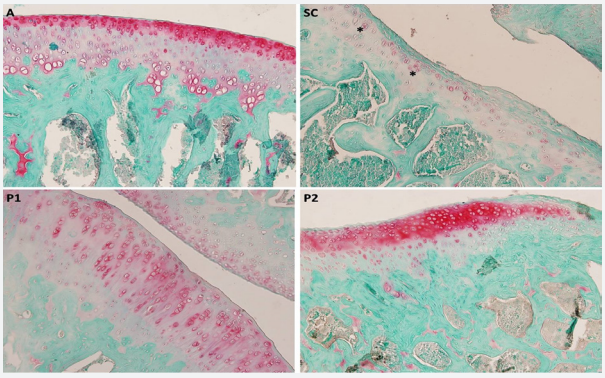

Fig. 1.

Table 1. Mankin scores of the groups.

Group/ Rat	Structure	Cells	Safranin O	Tidemark	Total
A-1	0	0	1	0	1
A-2	0	0	0	0	0
A-3	0	0	1	0	1
A-4	0	0	0	0	0
A-5	0	0	1	0	1
A -6	0	0	0	0	0
A -7	0	0	0	0	0
SC-1	3	3	2	0	8
SC-2	3	2	2	1	8
SC-3	5	2	2	1	10
SC-4	3	3	2	1	9
SC-5	3	3	2	0	8
SC-6	5	3	3	1	12
SC-7	3	3	2	0	8
P1-1	1	2	2	0	5
P1-2	1	2	1	0	4
P1-3	1	1	1	1	4
P1-4	1	2	2	1	6
P1-5	1	1	2	0	4
P1-6	2	1	2	0	5
P1-7	1	2	2	1	6
P2-1	3	1	1	1	6
P2-2	1	0	2	1	4
P2-3	2	2	2	1	7
P2-4	2	2	2	1	7
P2-5	1	3	1	0	5
P2-6	2	2	1	0	5
P2-7	1	2	2	0	5

A: Arthrotomy group
SC: Surgical control
P1:Propolis 100
P2 Propolis 200

4. Discussion

OA is a common degenerative disorder. It affects millions of people worldwide and usually causes workforce losses and decrease in the quality of life. The searches on the therapeutic treatment methods for OA aim at changing the course of the disease and decreasing the progression of cartilage degradation. MMPs and MMP inhibitors control the turnover and the function of the extracellular matrix. Therefore, they play a dominant role in the pathophysiology of OA (Pasternak and Aspenberg, 2009; Zhang et al., 2015).

Propolis is a substance that derived from plant resins by honeybees. It contains more than 300 different chemical compounds (Cardile et al., 2003; Guney et al., 2011). It has been shown in various studies that propolis has antioxidant, anti-inflammatory, immunomodulatory, antitumor and neuro-protective features. Also it has time dependent beneficial effects on fracture healing (Guney et al., 2011; Yüce et al., 2015).

PB is a flavonoid component of propolis (Zhang et al., 2015). PB inhibits the release of MMP-1 (Collagenase 1), MMP-3 and MMP-13 (Collagenase 3) from human chondrocytes. Thus, it reduces the facilitating effect of MMPs in the pathophysiology of OA. In addition to the inhibitory effect of PB on MMPs, it has also been reported to reduce inducible nitric oxide synthase (INOS), TNF-α, IL-1β and IL-6 release (Gao et al., 2010; Soromou et al., 2014; Zhang et al., 2015). This may contribute to the anti- inflammatory effect of propolis.

IL-1β is an important cytokine in cartilage tissue degradation. IL-1β increases the levels of INOS and Cyclooxygenase 2 (COX-2) with consequent increase of NO and arachidonic acid metabolites in chondrocytes. In particular, NO increases the release of MMPs from chondrocytes and inhibits the proteoglycan synthesis (Lyons-Giordano et al., 1993; Cipolletta et al., 1998; Cardile et al., 2003). CAPE is another flavonoid component of propolis. It reduces the deleterious effects of IL-1β and inhibits Xanthine oxidase activity on chondrocytes in cartilage tissue cultures (Cardile et al., 2003; Armutcu et al., 2015). Therefore, this significant effect of CAPE on IL-1β decrease the formation of free radicals and can explain the antioxidant properties of propolis. In addition, CAPE is a potent inhibitor of receptors that stimulate the activation of T cells that play a key role in inflammatory diseases. CAPE significantly inhibits IL-2 gene transcription that stimulates IL-2 and T cells (Marquez et al., 2004).

There were few studies that have been presented the protective action of propolis in human cartilage cultures (Cardile et al., 2003; Armutcu et al., 2015). On the contrary, to the best of our knowledge, this is the first study to report the protective effects of propolis in an experimental osteoarthritis model in rats. Rat medial meniscectomy model was chosen for achieving the progressive cartilage degeneration because an appropriate cartilage degeneration can be obtained as in human knee osteoarthritis in a period of four weeks (Bendele, 2001; Janusz et al., 2002).

The histological scores of both groups receiving propolis therapy in this study were significantly better compared to the SC group in terms of cartilage structure, chondrocyte status, amount of matrix staining with Safranin O and the integrity of tidemark. In other words, less experimentally induced cartilage degeneration occurred in the groups receiving

propolis compared to the control group. Also there were no statistically significant histological score difference between the P1 and P2 groups.

This study suggest that propolis has a decelerating effect on experimentally induced OA. The result exhibited by propolis under this experimental study can be explained by the effects of its active ingredients such as CAPE and PB on interleukins, TNF-α and MMPs. The fact that the findings are not supported by biochemical data is a weakness of this study.

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