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ORIGINAL ARTICLE

The effect of royal jelly on CD3$^+$, CD5$^+$, CD45$^+$ T-cell and CD68$^+$ cell distribution in the colon of rats with acetic acid-induced colitis

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Abstract
Background: Traditional medicines and health supplements have historically been used to treat many illnesses but most of them have not been evaluated objectively to prove their efficacy. We have been investigating the effects of royal jelly (RJ) supplements on acetic acid-induced colitis on the distribution of CD3$^+$, CD5$^+$, CD45$^+$ T-cell and CD68$^+$ cells in rats.
Methods: The rats were divided into four equal groups: control group, royal jelly-treated (RJ – 150 mg kg^{-1} body weight), acetic acid-treated (colitis) and acetic acid-treated (colitis) + royal jelly (CRJ – 150 mg kg^{-1} body weight). Colitis was induced by intracolonic instillation of 4% acetic acid; the control group received physiological saline (10 mL kg^{-1}). Colon samples were obtained under deep anaesthesia from animals in four groups. Tissues were fixed in 10% formalin neutral buffer solution for 24 h and embedded in paraffin.
Results: The proliferative response of CD3$^+$ and CD45$^+$ T cells stimulated with colitis was affected by colitis treated with RJ. No differences were found in CD5$^+$ T cells and CD68$^+$ macrophages in the colitis treated with RJ.
Conclusions: This study has shown that RJ has anti-inflammatory and cell regeneration effect in the colon of rats with acetic acid induced colitis.

Introduction

Mucosal surfaces of the intestinal tract represent one of the main routes for microbial pathogens to enter a host and are important sites of microbially induced diseases. The intestinal immune system is responsible for protecting a large surface area from invasion by pathogens,

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whilst remaining tolerant to a complex microflora and to dietary Ags.[1] Pathogens might lead to acute inflammation, characterised by an excessive production of inflammatory mediators. These are responsible for the recruitment of specific cell types, for example, lymphocytes, macrophages, or neutrophils.[2]

A characteristic feature of the immune system at the mucosal surfaces, such as the respiratory and intestinal tracts, is that protective, cell-mediated and humoral immune responses against invading pathogens are allowed to proceed whilst pathogenic responses against innocuous antigens are prevented. The importance of an intact immune system for intestinal homeostasis is shown by the fact that a number of immune manipulations, including the deletion of cytokine genes and alterations in T lymphocytes subsets, lead to the development of an inflammatory bowel disease-like syndrome in mice.[3] A number of infiltrated lymphocytes, neutrophils, macrophages and natural killer cells are significantly increased after injury induced by toxins such as alcohols, CCl_4, and acetic acid and most of which causes activation of lymphocytes, neutrophils, monocytes and macrophages.[4] The activation of these cells causes apoptosis, necrosis and inflammations with release to cell death ligand.[5,6] Following damage and inflammation in cell tissue, repair by anti-inflammatory agents takes place in necrotic and apoptotic cells.[6]

Natural compounds, such as components of Royal Jelly (RJ), can play a significant role against inflammation, cancer,[7] colitis,[8] liver damage,[9] immunomodulatory,[10] cell proliferation,[11] and hepatocyte and tubular cell apoptosis,[12] RJ that is produced by the hypopharyngeal and mandibular glands of worker honeybees contains a variety of free amino acids, vitamins and sugars. A number of biological and immunoregulatory effects attributed to RJ have been reported.[13] In addition, Şimşek et al.[14] reported that RJ might be used as a supportive agent in immune deficiency and anaemic patients, because of both the increased erythrocyte count and the diameter.

The aim of this study was to evaluate the protective and antioxidative effect of RJ in acetic acid-induced colitis by determining changes in distribution of T-lymphocytes and macrophages.

Material and methods

Twenty-four adult, Wistar, albino rats ($n = 6 \times 4$) weighing about 230–300 g were obtained from the Laboratory of Animal Science, Medical School, Firat University, Elazig, Turkey. The animals were given standard rat pellets (Van Feed Factory, Van, Turkey) and tap water *ad libitum* and were housed in individual cages (360 mm × 200 mm × 190 mm), each containing three animals, for seven days prior to the start of the experiment.

The rats were divided into four equal groups. Control group, royal jelly-treated (RJ), acetic acid-treated (colitis) and acetic acid-treated (colitis) + royal jelly (CRJ). Control group received normal food and water during the experiment. The RJ group received normal food and royal jelly (150 mg kg^{-1}) suspended in water. The Colitis group received normal food and a dose of acetic acid dissolved in 0.9% NaCl delivered via the anus to the colon. The CRJ group received normal food along with 150 mg kg^{-1} royal jelly suspended in water and a dose of acetic acid dissolved in 0.9% NaCl delivered via the anus to the colon. The RJ used was purchased from a local natural food store in Istanbul, Turkey. All animals were housed in stainless steel cages under standard laboratory conditions (light period 07.00 h to 20.00 h, 21 ± 2 °C, relative humidity 55%), and received humane care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institute of Health.

All experimental animals were not fed for 24 h before induction of colitis. Each rat was lightly anesthetised with ether, and a polyethylene cannula was inserted into the lumen of the colon via the anus. The tip of the cannula was positioned at 8 cm proximal to the anus. Either 1 mL of acetic acid (4% vol/vol in 0.9% NaCl) or saline as the inert control was slowly infused into the distal colon. After 30 s exposure, 1 mL of saline (0.9%) was instilled in order to withdraw the previous solution from the colon.[8,15]

One month after initiation of the experiment, rats were euthanized under ether anaesthesia and the colon of each was excised and gently rinsed under tap water. The colon was then stretched on a piece of cork with mucosal surface upwards in the standard position for macroscopic examination and the severity of colitis was scored with the help of a magnifying glass. Finally, the colon was dissected and fixed in 10% formalin neutral buffer solution at room temperature for 24 h and cross-sections were processed routinely for light microscopy. Sections of 5 μm were stained with Mallory's triple stain and examined under a light microscope.

Colon tissue samples were fixed in 10% formalin neutral buffer solution, embedded in paraffin, and cut in 5 μm sections. CD3, CD5, CD45 and CD68 positive cells were determined with streptavidin–biotin–peroxidase staining method. Antigens were retrieved in deparaffinised and rehydrated sections by boiling in citrate buffer (10 mM, pH 6) for 15 min. T lymphocytes (CD3, CD5 and CD45) and monocyte/macrophage lineage cells (CD68) were determined with streptavidin–biotin–peroxidase staining method. For immunohistochemistry examinations, anti-CD3, anti-CD5, anti-CD45 (Abbiotec – USA) and monoclonal mouse anti-CD68 (Clone KP1, Invitrogen, 08-0125) primary antibodies and biotinylated secondary antibody (DAKO-Universal LSAB Kit-K0690) were used. The binding sites of antibody were covered with 3,3′-diaminobenzidine (Sigma) or aminoethylcarbazole substrate kit (AEC kit; Zymed Laboratories) and evaluated by high-power light microscopic (Nikon i50 or Optiphot 2, Japan). For each specimen, $CD3^+$, $CD5^+$, $CD45^+$ and $CD68^+$ immunoreactivity was determined in ten randomly selected areas using an approximately X20 objective lens.

Results

Light microscopy examination of the colon mucosa of control rats and RJ rats stained with Mallory's triple stain showed normal mucosal glands. The rats from the acetic acid-induced colitis group exhibited severe damage to the colon mucosa, with pathological changes including massive destruction of the epithelial layer and cellular debris,