Acta Histochemica 118 (2016) 588–595

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

The dual anti-inflammatory and antioxidant activities of natural honey promote cell proliferation and neural regeneration in a rat model of colitis

Hanaa Z. Nooh*, Nermeen M. Nour-Eldien

Anatomy & Embryology Department, Faculty of Medicine, Menoufia University, Egypt

ARTICLE INFO

Article history:
Received 8 April 2016
Received in revised form 16 June 2016
Accepted 20 June 2016

Keywords:
Ulcerative colitis
DSS
Cytokine
Oxidative stress
Honey
Immunohistochemistry

ABSTRACT

A decreased antioxidant capacity and excessive inflammation are well-known features in the pathogenesis of ulcerative colitis (UC). Recent evidence has suggested a role of honey in reducing colitis-induced inflammatory and oxidative stress markers. In this study, we examined whether the anti-inflammatory and anti-oxidative properties of honey have a beneficial effect on the enteric innervation and cellular proliferation of UC in rat. The colitis was induced in rats by dextran sodium sulphate (DSS). The effect of natural honey on induced colitis was assessed by the following parameters in colonic samples: tissue injury, inflammatory infiltration, interleukin-1β and -6, superoxide dismutase and reduced glutathione. In addition, the expression of tumour necrosis factor-α, inducible NO synthase, caspase-3, CD34, Ki67, S100, c-kit, and neuron-specific enolase were examined by immunohistochemistry. Compared to the DSS-induced colitis group, the honey-treated group had significantly improved macroscopic and microscopic scores and exhibited the down-regulation of oxidative, inflammatory, and apoptotic markers. In addition, up-regulation of intrinsic muscular innervation and epithelial cellular proliferation markers was detected. These results provide new insight into the beneficial role of natural honey in the treatment of DSS-induced colitis via the inhibition of colonic motor dysfunction and the inflammatory-oxidative-apoptotic cascade. In addition, the role of honey in epithelial regeneration was clarified.

1. Introduction

Ulcerative colitis is one of the inflammatory bowel diseases (IBD) that has a chronic course and relapsing bouts. The latter is responsible for fibrosis formation and uncontrollable pharmacological alterations that make surgical colonic resection is inevitable (Rieder et al., 2012).

Although the etiology of ulcerative colitis is a matter of controversy, the pivotal roles of inflammatory and oxidative stress in the pathogens of ulcerative colitis are established (Cetinkaya et al., 2005, 2006). Recently, researchers correlated the occurrence of inflammatory and fibrotic processes, in colitis, to colonic neuromuscular compartment alternation (Bernardini et al., 2012; Ippolito et al., 2015). Inhibition of epithelial apoptosis was considered as a crucial factor in the healing process of chronic ulcerative colitis (Goretsky et al., 2012).

New trends to use a variety of natural products that safely suppress the pro-inflammatory pathway and control IBD has been observed (Debnath et al., 2013; Yang et al., 2014; Algieri et al., 2014). From ancient times, the honey has been used for accelerating the wound healing process (Molan, 1998, 1999a). Honey is a viscous liquid that prepared by bees from the nectar of different flowers and plants. It is a healthy, natural, energy producing and easily digestible food. More than 181 substances are found in raw natural honey. It contains a blend of vitamins, minerals, enzymes, antioxidants, protein, carbohydrate and lipids (Bloggers, 2015). It is considered as an effective agent for the treatment of ulcers, bed sores and other skin infections accompany with burns and wounds (Molan 1999b; Blassa et al., 2006). Also, it heals abscesses and deep infected surgical wounds, especially those, do not respond to the usual therapy (Kassim et al., 2010; Hussein et al., 2011; Yao et al., 2011). Its healing activity is through enhancing new tissue growth through prevention of infection (Bilsel et al., 2002; Medhi et al., 2008). Honey was effective in treatment of ulcerative colitis induced by trinitrobenzene sulfonic acid (Bilsel et al., 2002)

* Corresponding author at: Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
E-mail addresses: hanaa.nouh@med.menofia.edu.eg, drhanaanooh@gmail.com, hanaa_noh_10@yahoo.com (H.Z. Nooh).

http://dx.doi.org/10.1016/j.acthis.2016.06.006
0065-1281/

and acetic acid (Mahgoub et al., 2002). The DSS-induced colitis model is a well appreciated and widely used model of inflammatory bowel disease because of its simplicity in induction of the three disease forms (acute, chronic, or relapsing). Also, DSS colitis has many similarities to human IBD, the chronic phase always represented with dysplasia as clinical course in human (Kanneganti et al., 2011). In addition, in a genetically engineered mice, different molecules involved in the mediation of inflammation and oxidation factors could be detected in DSS colitis. Moreover, DSS induced defects in the epithelial barrier integrity, through direct toxic, apoptosis and anti-proliferative effects to colonic epithelial cells (Perše and Cerar, 2012). The well-known anti-inflammatory and antioxidant capacities of honey may be useful for the prevention of chronic inflammatory process as, diabetes mellitus; cardiovascular diseases; atherosclerosis; etc. (Vallianou et al., 2014). Polyphenol constituents of natural honey counter oxidative stress, attenuate the microglia-induced neuroinflammation in addition to its impact on many neurodegenerative diseases, neuronal apoptosis, necrosis, synaptic plasticity (Rahman et al., 2014). Through mobilization of endogenous stem cells, recent researches proved the regenerative effect of natural honey on the degenerated tissue as; intestinal, ovarian (Prasetyo and Erma Safitri, 2016) and testicular (Safitri et al., 2016). Also, the Honey bee venom has positive effect on neuronal cell differentiation and proliferation (Kouchesfahani et al., 2010).The aim of this work is to demonstrate if anti-inflammatory anti-oxidant properties of the honey have a beneficial effect the anti-inflammatory and anti-oxidative properties of honey have a beneficial effect on enteric innervation and cellular proliferation on dextran sodium sulfate (DSS) – induced ulcerative colitis in the rat.

2. Material and methods

2.1. Chemical

DSS salt; a product of leu co nostoc spp, Sigma-Aldrich (St Louis, MO, USA), was available in the form of powder. (Mw > 500 kDa) that contains 0.5–2% phosphate buffer. It was dissolved in drinking water (Egger et al., 2000).

Honey used in this study is a wild multifloral honey supplied by frame of Faculty of Agriculture, Menoufia University, Egypt. The composition of this honey is presented as follows: total reducing sugar (77.7%) [Fructose (42.14%), glucose (28.19%), maltose (4.2%), sucrose (3.17%) and 5-hydroxymethyl-2-furfural].

2.1.1. Honey supplementation

The prescribed honey dosage was based on previous pilot study as; we gave natural unprocessed bee honey at dose of 0.5, 0.75 and 1 g/rat, (once/day per day by oral gavage) respectively. Treatment with 1 g/rat (5 g/kg) significantly attenuated the extent and severity of the histopathologic and biochemical features of ulcerative colitis rats and this effects was less promising in the other two doses, 0.5, and 0.75. So, we used only the effective dose, 1 g/rat, i.e. 5 g/kg in this study. This effective dose was previously prescribed by Mahgoub et al. (2002).

2.2. Animals

For studying the effect of natural honey ton the chronically induced UC, forty healthy (40) adult male albino Wistar rats, weighing 180 ± 30 g, were housed in a temperature conditioned room (24 ± 2 °C) in separate cages for at least one week before and through the experimental work, being maintained on a standard diet (composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitamins) (National Research Center Egypt). Water was available ad-libitum. The study protocol was in accordance with the guideline for animal research and approved by the Ethical Committee of a Faculty of Medicine, Menoufia University, Egypt.

Table 1
Scoring of the disease activity index (DAI).

Score	Weight changes (%)	Stool consistency	Occult/gross rectal bleeding
0	1	Normal[a]	Normal
1	1–5	Normal	Occult blood +
2	5–10	Loose stool[b]	Occult blood ++
3	10–15	Loose stool	Occult blood +++
4	>15	Diarrhea[c]	Gross bleeding

[a] Normal stool: well formed pellets.
[b] Loose stools: pasty and semi-formed stools which do not stick to the anus.
[c] Diarrhea: liquid stools that stick to the anus. The total clinical score was calculated by summation of the previous parameters. It was classified as: mild activity: ranged from 1 to 4, moderate activity: 5–8, & maximal activity: 9–12.

2.3. Study design

Forty rats were randomly and equally divided into four groups:

Group I (negative control group, n = 10): Left without treatment and served as the untreated control group.

Group II (sham control group, n = 10): received 1 g/rat of natural unprocessed honey orally once/day for three weeks.

Group III (colitis induced group, n = 10): The colitis was induced by adding DSS to drinking water; for three weeks (5% for one week followed by 3% for two weeks) (Moreau et al., 2002).

Group IV (treated group, n = 10): They received the previous described dose of natural honey, once/day for three weeks after induction of colitis.

All rats were weighed at the beginning and the end of the experiment and Weight variation% was calculated. At the end of the experiment, the rats were sacrificed by cervical decapitation and the last 8 cm of the colon was excised, opened longitudinally and rinsed with saline solution.

2.4. Morphological (clinical) assessment

To determine the clinical activity of DSS colitis, we examined the rats for percentage weight change (calculated compared with that on Day 0), stool consistency, occult and/or bleeding were evaluated and scored every week during the experimental period. The summation of the three parameter described earlier by Cooper et al. (1993) as disease activity index (DAI) (Table 1).

2.5. Macroscopic and microscopic study

After washing the mucosa, mucosal injury was assessed macroscopically using the grading scale by Morris et al. (1989), a score of 0 = no damage, 1 = localized hyperemia but no ulcers, 2 = linear ulcers with no significant inflammation, 3 = linear ulcer with inflammation at 1 site, 4 = ulceration and inflammation at 2 or more sites

Colon specimens of 10 animals were fixed in 10% neutral formol saline for 24 h, dehydrated in ascending grades of alcohol, cleared and embedded in paraffin. Sections of 5 μm thick were cut by microtome, and subjected to H&E stain (Kiernan, 1981) and immunohistochemical staining. For each animal, five (5) randomly taken hematoxylin and eosin slides were examined and scored for histopathological evaluation. The slides were coded to prevent observer bias during evaluation. All tissue sections were examined in a blinded fashion by experienced