To evaluate the effect of different doses of Manuka honey in experimentally induced inflammatory bowel disease in rats. Adult Wistar rats of either sex were used (n = 30). Colitis was induced by a single intracolonic administration of TNBS dissolved in 35% ethanol. The rats (n = 30) were divided into five groups (n = 6) and were treated with vehicle (ethanol), TNBS, Manuka honey (5 g/kg, p.o.), Manuka honey (10 g/kg, p.o.) or sulfasalazine (360 mg/kg, p.o.) body weight for 14 days. After completion of treatment, the animals were killed and the following parameters were assessed: morphological score, histological score and different antioxidant parameters.

Manuka honey at different doses provided protection against TNBS-induced colonic damage. There was significant protection with Manuka honey 5 g/kg as well as with 10 g/kg body weight compared with the control (p < 0.001). All the treated groups showed reduced colonic inflammation and all the biochemical parameters were significantly reduced compared with the control in the Manuka honey treated groups (p < 0.001). Manuka honey at different doses restored lipid peroxidation as well as improved antioxidant parameters. Morphological and histological scores were significantly reduced in the low dose Manuka honey treated group (p < 0.001). In the inflammatory model of colitis, oral administration of Manuka honey 5 g/kg and Manuka honey 10 g/kg body weight significantly reduced the colonic inflammation. The present study indicates that Manuka honey is efficacious in the TNBS-induced rat colitis model, but these results require further confirmation in human studies. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: antioxidant defence system; Manuka honey; colitis; inflammatory bowel disease.

INTRODUCTION

Inflammatory bowel disease is an idiopathic, chronic inflammatory condition, which affects the gastrointestinal tract. The incidence of inflammatory bowel disease in Western countries is 11 cases of ulcerative colitis per 100 000 population and 7 cases per 100 000 population of Crohn’s disease, but currently the incidence of each are estimated to be about equal. It has been suggested that intestinal damage in inflammatory bowel disease (IBD) is related both to increased free radical production resulting from respiratory burst of infiltrating phagocytic cells and to impaired antioxidant defence (D’Odorico et al., 2001). The protective functions of phagocytic cells are mediated by the toxic inflammatory mediators that are released. Inflammatory mediators, including reactive oxygen species (ROS) and cytokines, contribute to the inflammatory cascade in modulating the immune system of IBD (Murata et al., 1995; Nieto et al., 2000; Wendland et al., 2001). Pathophysiological changes in inflammatory bowel disease are well established. One of the mechanisms is through cytokines that are secreted from macrophages such as TNF-α, IL-1 and IL-8. TNF-α stimulates and induces the production of other inflammatory mediators such as ROS, and it also activates oxidative stress-responsive genes which amplify and prolong inflammation during IBD (Larrick and Wright, 1990). Growing evidence also demonstrates the significance of oxidative stress both in the clinical and experimental studies of IBD. The increase of ROS and the impairment of antioxidant defence mechanisms were postulated to be causative factors in inflammatory diseases (Han and Meydani, 2000). Under physiological conditions, a fine balance is maintained between the oxidant and antioxidant systems but it is impaired in pathological circumstances such as IBD. Because of increased oxidative stress, the antioxidant system becomes insufficient and the susceptibility of target molecules to oxidative stress increases. Oxidant/antioxidant balance has been suggested to be an important factor for initiation and progression of cancer (Miranda et al., 2000). Excessive production of ROS in mucosal cells induced by inflammatory and immune responses could directly or indirectly cause damage to intestinal epithelial cells, subsequently influence the mucosal integrity or initiate an inflammatory signaling cascade and lead to severe impairment in experimental colitis (Oz et al., 2005; Kurutas et al., 2005).
A number of drugs are available for the treatment of ulcerative colitis. Drugs such as 5-aminosalicylic acid (5-ASA), sulfasalazine, and glucocorticoids could inhibit the inflammatory mediators through different mechanisms which are engaged in the down-regulation of the immune and inflammatory responses of IBD, their adverse reactions during prolonged treatment and the high relapse rate limit their use (Joshi et al., 2005; Auphan et al., 1995).

There are several herbal products including honey that showed antioxidant properties in various disease conditions (Bora et al., 2007). Additionally, honey of various origins was evaluated for its analgesic, anti-inflammatory, and ulcerogenic properties alone and in combination (Azim et al., 2007; Mythilypriya et al., 2007; Lusby et al., 2006). The present study includes Manuka honey which is obtained from the plant Leptospermum scoparium and used therapeutically for its antibacterial properties. Based on earlier studies, it is suggested that Manuka honey has various biological activities and might have different pharmacological effects including wound healing, fungal infections, ophthalmology, diabetes, gastrointestinal tract, skin ulcers and infections. Honey is a popular natural sweetener and a powerful antioxidant used in the day to day life. Varieties of enzymes are present in honey such as oxidase, invertase, amylase, catalase etc. So the present study was undertaken to evaluate the ameliorative effect of different doses of Manuka honey in a TNBS-induced colitis model of IBD and to analyse its effects on MPO, lipid peroxidation, glutathione level and different antioxidant enzymes including morphological and histological parameters.

MATERIALS AND METHODS

Materials. Hexadecyl-trimethylammonium bromide buffer (HTAB), O-dianisidine dihydrochloride, glutathione reductase, nicotinamide adenine dinucleotide phosphate reduced (NADPH) and thiobarbituric acid (TBA) were purchased from Sigma Chemical (St Louis, MO, USA). Phosphate buffered saline (PBS), Tris-HCl buffer and ethylenediamine tetraacetic acid (EDTA) were purchased from M/s HiMedia Chemicals (Mumbai, India). Reduced glutathione, hydroxylamine hydrochloride, trichloroacetic acid, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and nitroblue tetrazolium (NBT) were procured from M/s Sisco Research Laboratories Pvt Ltd (Mumbai, India). The pellet diet duly approved by the Institute’s Animal Ethics Committee was obtained from M/s Ashirwad Industries (Punjab, India).

Animals and treatment. The experiments performed on pathogen-free young male Wistar rats weighing 150–250 g after the study were cleared by the Institute’s Animal Ethics Committee. The animals were obtained from the Central Animal House of the Postgraduate Institute of Medical Education and Research, Chandigarh, India. The animals were divided into five groups containing six animals in each group. Group I (ethanol as vehicle), Group II (TNBS 20 mg in 35% ethanol, applied through the rectum as a single intracolonic application into descending colon through a rubber catheter, Group III (sulfasalazine 360 mg/kg b.wt as a positive control), Group IV (Manuka honey at a dose of 5 g/kg b.wt) and Group V (Manuka honey at a dose of 10 g/kg b.wt). The animals were given the different doses of honey for 2 weeks. The animals were allowed free access to water and a normal pellet diet. They were housed in polycarbonate cages bedded with sterilized rice husks in at 12 h light and dark cycle.

Induction of colitis. Male Wistar rats weighing 150–250 g were used for the induction of colitis. Animals received humane care and the experimental protocol complied with the guidelines of our institution. Colitis was induced according to a previously described method (Morris et al., 1989). Animals were anaesthetized with ethyl ether after which a single intracolonic application of 20 mg of TNBS dissolved in 0.25 mL of either 35% ethanol in saline (v/v) or saline, referred to as TNBS-ethanol or TNBS-saline, respectively, into the descending colon was given. The control rats received either the same volume of 35% ethanol diluted with saline (v/v) or just saline. The animals were killed 14 days after TNBS-ethanol administration and the colonic mucosa was obtained for evaluation of damage microscopically as well as the antioxidant status.

Tissue preparation. The tissues were excised and perfused with ice-cold perfusion solution (0.15 m KCl, 2 m EDTA, pH 7.4). The tissues were homogenized in Tris-HCl buffer (50 mM, pH 7.4), and the homogenates were centrifuged at 10 000 × g at 4 °C for 30 min to obtain a post-mitochondrial supernatant (PMS). The PMS was used for the estimation of the antioxidant defence system.

Myeloperoxidase activity (MPO). Myeloperoxidase activity was measured according to the method of Krawiesz et al. (1984). The tissue samples were homogenized in 50 mmol/L potassium phosphate buffer (pH 6.0). The homogenate was centrifuged at 10 000 × g for 15 min at 4 °C. The supernatant was discarded and the pellet was resuspended in hexadecyl-trimethylammonium bromide buffer (HTAB 0.5% w/v in 50 mmol/L potassium phosphate buffer, pH 6.0). The suspension was sonicated on ice, and again centrifuged at 12 000 × g for 15 min at 4 °C. Supernatants were diluted in potassium phosphate buffer (pH 6.0) containing 0.167 mg/mL of O-dianisidine dihydrochloride and 0.0005% of H2O2. Changes in the absorbance at 450 nm, every 10 s over 2 min, were recorded with a spectrophotometer (Spectronix Genesys 2, Rochester, New York, USA). One unit of MPO activity was defined as the quantity of MPO degrading 1 μmol H2O2/min/mg protein at 25 °C.

Lipid peroxidation. Lipid peroxidation was estimated by the method of Ohkawa et al. (1978) in tissue homogenates. Briefly, the reaction mixture contained Tris-HCl buffer (50 mM, pH 7.4), ter-butyl hydroperoxide (BHP) (500 μM in ethanol) and 1 mM FeSO4. After incubating the samples at 37 °C for 90 min, the reaction was stopped by adding 0.2 mL of 8% sodium dodecyl sulphate (SDS) followed by 1.5 mL of 20% acetic acid (pH 3.5). The amount of malondialdehyde (MDA) formed during incubation was estimated by adding 1.5 mL of 0.8% TBA and further heating the mixture at 95 °C for 45 min. After cooling, the samples were centrifuged, and the TBA reactive substances (TBARS)
Reduced glutathione (GSH). Reduced glutathione estimation in the tissue homogenate was performed by the method of Paglia and Valentine (1967). The required amount of tissue homogenate was mixed with 25% of trichloroacetic acid and centrifuged at 2000 × g for 15 min to settle the precipitated proteins. The supernatant was aspirated and diluted to 1 mL with 0.2 M sodium phosphate buffer (pH 8.0). Later, 2 mL of 0.6 M DTNB was added. After 10 min the optical density of the yellow-coloured complex formed by the reaction of GSH and DTNB (Ellman’s reagent) was measured at 405 nm. A standard curve was obtained with standard reduced glutathione. The levels of GSH are expressed as μg GSH/mg tissue.

Superoxide dismutase (SOD). Superoxide dismutase was estimated according to the method of Kono (1978). Briefly, the reaction mixture containing solution A (50 mM sodium carbonate, 0.1 mM EDTA, pH 10.0), solution B (96 μM nitroblue tetrazolium (NBT) in solution A) and solution C (0.6% Triton X-100 in solution A) were incubated at 37 °C for 10 min. The reaction was initiated by adding 100 μL solution D (20 mM hydroxyamine hydrochloride, pH 6.0). The rate of NBT dye reduction by O$_2^-$ anion generated due to photoactivation of hydroxylamine hydrochloride was recorded at 560 nm in the absence of PMS. Later, small aliquots of PMS were added to the reaction mixture and 50% inhibition in the rate of NBT reduction by SOD present in the enzyme source was recorded. One unit of enzyme activity was defined by the 50% inhibition of NBT. The levels of SOD are expressed in terms of IU/mg protein.

Catalase (CAT). Catalase activity was measured in the PMS by the method of Luck (1963). The final reaction volume of 3 mL contained 0.05 M Tris-buffer, 5 mM EDTA (pH 7.0) and 10 mM H$_2$O$_2$ (in 0.1 M potassium phosphate buffer, pH 7.0). About 50 or 100 μL aliquots of the tissue PMS was added to the above mixture. The rate of change of absorbance per min at 240 nm was recorded. The level of CAT is expressed in terms of μmol H$_2$O$_2$ consumed/min/mg protein.

Glutathione peroxidase (GPx). The GPx activity was measured by the method of Paglia and Valentine (1967). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide, 0.2 mM NADPH, 1 U glutathione reductase and 1 mM reduced glutathione. The sample, after its addition, was allowed to equilibrate for 5 min at 25 °C. The reaction was initiated by adding 0.1 mL of 2.5 mM H$_2$O$_2$. The absorbance at 340 nm was recorded for 5 min. The levels of GPx were expressed in terms of nmol NADPH consumed/min/mg of protein using the extinction coefficient of 6.2 × 10$^4$ M$^{-1}$ cm$^{-1}$ at 340 nm.

Assessment of inflammation. The rats were killed under ether anaesthesia by cervical dislocation 2 weeks after the TNBS administration. The distal 10 cm of the colon was dissected longitudinally into three pieces for morphological analysis, histological analysis and antioxidant assay.

Morphological analysis. The gross inflammatory index was assessed visually for inflammation according to the enteritis gross morphological score (Vogel and Goethe, 2002): 0, no sign of inflammation in the whole 10 cm length of intestine; 1, slight inflammation, slight redness, villi visible under 15-fold magnification; 2, intermediate inflammations, discontinuous intermediate redness of villi; 3, intensive inflammation, hyperaemia, intensive redness of villi.

Histological analysis. Microscopic examinations were done by a qualified blinded pathologist using haematoxylin and eosin staining in a blinded fashion as described by Levine et al. (2002), in the group treated with TNBS in 35% ethanol and ethanol only treated group and also in other groups after pharmacological intervention using the following score: 0, normal; 1, mild mixed infiltrates in the lamina propria; 2, focal superficial ulceration of the mucosa only, moderate cryptitis and crypt abscess; 3, deep ulceration penetrating colonic wall through mucosa till muscularis mucosa and severe inflammation; 4, necrosis through large bowel wall.

Statistical analysis. The statistical significance of differences between the various groups was determined by using one-way analysis of variance (ANOVA) followed by the post-hoc test Bonferroni test, Dennett t (2 sided) and Tukey test used for data analysis with respect to the control. Values of $p < 0.05$ were considered significant.

RESULTS

The assessment was done by gross morphology and microscopic histopathology studies together with various biochemical parameters including myeloperoxidase, lipid peroxidation, superoxide dismutase, catalase, glutathione peroxidase activities and reduced glutathione.

During the study there was no mean weight loss/gain and none of the rats were excluded from the study for any reason. More than 50% of animals in the groups had diarrhoea after the induction of inflammation in the colon. Although the rats continued to have diarrhoea for 6–7 days, in the treatment group the diarrhoea were obsolete. After the rats were killed and the colons opened, it was seen that some of the animals had local peritonitis compatible with transmural necrosis and inflammatory masses in the region of the descending colon.

Morphological findings

Fourteen days after TNBS treatment, the morphology of the colon revealed inflammatory change in the mucosa. The gross inflammatory index along the 10 cm length of colon was assessed for inflammation according to the scoring system. The morphological score in the control group animals was significantly lower ($p < 0.001$) compared with the TNBS groups (Fig. 1).
Manuka honey (5 mg/kg, LMH) treatment significantly reduced \( (p < 0.05) \) the morphological inflammatory changes compared with the TNBS group animals (Fig. 1). Also, Manuka honey (10 mg/kg) (HMH) was effective in treating the morphological changes induced by TNBS (Fig. 1).

**Histological findings**

The microscopic examination of the colon tissue showed the presence of inflammation in the mucosa which was scored as described previously by Levine *et al.* (2002). In the control group, there was no significant change \( (p < 0.001) \) at the cellular level, however, reactive lymphoid follicles were present (Fig. 2). The scanner view of the tissue shows intact mucosa inflamed lamina propria and widened submucosa. There was significant change in the vehicle treated group and the examination of tissue showed mixed inflammation rich in neutrophils and eosinophils in the lamina propria (Fig. 3). There was also presence of lymphocytes infiltration into the crypt with features of cryptitis (Fig. 4a, b). These findings indicated severe inflammation of the colon and the histological score was very high compared with
the vehicle treated group. In Manuka honey group the tissue showed mild inflammation of lamina propria by lymphocytes and scattered eosinophils. There was no evidence of cryptitis and no crypt abscess was seen compared with the control (Figs 5, 6, 7). There was mild inflammation and lamina propria with occasional neutrophils. No cryptitis was found in this group. The histological score was equal for both doses of Manuka honey.

Myeloperoxidase activity (MPO)

There was a 6-fold increase ($p < 0.001$) in the myeloperoxidase activity after 14 days of TNBS treatment compared with the control. Treatment with Manuka honey (5 mg/kg) and 10 mg/kg significantly reduced the TNBS-induced myeloperoxidase activity 2 fold. Although there were significant decreases ($p < 0.05$) in TNBS-induced MPO activity in all the treated groups, Manuka honey at a dose of 10 mg/kg, p.o. significantly decreased ($p < 0.001$) the MPO activity reaching the control levels (Fig. 8).

Lipid peroxidation

TNBS treatment significantly increased the lipid peroxidation levels by 2 fold compared with the control. Treatment with Manuka honey (5 mg/kg) significantly decreased the LPx levels ($p < 0.05$) to their control values, whereas Manuka honey (10 mg/kg) showed less effectiveness compared with the low dose treatment in decreasing the lipid peroxidation levels (Fig. 9).

Superoxide dismutase activity

TNBS treatment significantly ($p < 0.001$) decreased the SOD activity by 2 fold compared with the control, whereas with either Manuka honey (5 mg/kg) or
Effect of different doses of Manuka honey (MH) on the lipid peroxidation after 14 days following single intracolonic administration of TNBS-induced colitis in rats. Values are mean ± SD, n = 6, * p < 0.05 considered significant with respect to the control, # p < 0.05 considered with respect to TNBS group. (LMH, low dose Manuka honey (5 g/kg, p.o.); HMH, high dose Manuka honey (10 g/kg, p.o.); SSZ, sulphasalazine; TNBS, trinitrobenzene sulphonic acid).

Effect of different doses of Manuka honey (MH) on the superoxide dismutase activity after 14 days following single intracolonic administration of TNBS-induced colitis in rats. Values are mean ± SD, n = 6, * p < 0.05 considered significant with respect to the control, # p < 0.05 considered with respect to TNBS group. (LMH, low dose Manuka honey (5 g/kg, p.o.); HMH, high dose Manuka honey (10 g/kg, p.o.); SSZ, sulphasalazine; TNBS, trinitrobenzene sulphonic acid).

Catalase activity

In the Manuka honey (10 mg/kg) group, catalase activity was comparatively higher than the Manuka honey 5 mg/kg groups (p < 0.05) but significantly lower than the control group (Fig. 11). Catalase activity in the TNBS group was significantly decreased (p < 0.001) by 1.5 fold compared with the control. Treatment with Manuka honey (5 mg/kg) significantly increased the TNBS-mediated decreased CAT activity.

Glutathione peroxidase activity

TNBS treatment significantly decreased the GPx activity by 3 fold. Treatment with Manuka honey (5 mg/kg) and Manuka honey (10 mg/kg) significantly increased (p < 0.05) the TNBS-mediated decreased GPx activity reaching almost the control levels (Fig. 12).

Reduced glutathione

Compared with the control group TNBS treatment significantly decreased the GSH level (p < 0.001). After 14 days in all treated groups (LMH and HMH) there was a significantly (p < 0.05) increased glutathione reductase level at the site (Fig. 13).

DISCUSSION

The trinitrobenzene sulphonic acid (TNBS) colitis model in rats is a well established experimental model for IBD in rats. Rectal application of TNBS which is dissolved in 35% ethanol produces acute colonic inflammation followed by chronic inflammation. TNBS may induce colitis by different mechanisms. It binds covalently...
to the E-amino acid group of lysine and thus it can covalently modify cell surface proteins. Pre-sensitized T lymphocytes can lyse haptan modified autologous cells quite efficiently. Whereas T lymphocytes will lyse haptan modified autologous cells only if the animal has been pre-sensitized, macrophages will destroy TNBS modified autologous cells in the absence of pre-sensitization. The initial local inflammation followed by development of chronic inflammation is due to activation of the immune system. The colitis produced by this experimental method mimics the similar disease condition evidenced in human inflammatory bowel disease. This standard experimental model is well established for inflammatory bowel disease (Nieto et al., 1998a, 1998b; Kruidenier and Verspaget, 1998; Grisham et al., 1991). This suggests that TNBS-induced colitis may partially be mediated by cytotoxic reactive oxygen metabolites (The et al., 1978; Kunin and Gallily, 1983). Other sources of TNBS-induced ROS production by the inflamed colonic mucosa include the mitochondrial and microsomal transfer chains, xanthine oxidase, cyclooxygenase and lipooxygenase enzymes (Karayalcin et al., 1990; Schumbert et al., 1989; Sedor, 1986; Slater, 1984). ROS can also be produced by the phagocytic cells through the NADPH pathway and by the overproduction of proinflammatory mediators (i.e. cytokines and arachidonate metabolites) (Karayalcin et al., 1990). It has been suggested that neutrophils are predominantly responsible for the production of excessive amounts of ROS by the tissue in colonic inflammation (Hermanowicz et al., 1985; Wallace et al., 1998). MPO, a constitutive component of neutrophil azurophilic granules, is a good marker of inflammation and tissue injury (Nieto et al., 1998b). A six-fold increase in MPO activity was observed after TNBS treatment. The decrease in MPO activity after Manuka honey treatment with different doses could be explained by a reduction in the number of neutrophils within the mucosa, which would then decrease the risk of hypochlorous acid damage as evident even from the histological studies. The LM was equally effective to the HMH dose. This leads to the overall concept of attempting to restore normal homeostasis.

Moreover, it is evident from our study that the functional status of antioxidant defence system in the colonic epithelial cells of rats challenged with TNBS is impaired (Zea-Iriarte et al., 1996). The efficacy of the antioxidant defence system may be impaired during inflammation. It has also been described earlier that the mammalian colon has only small amounts of antioxidant enzymes such as CAT, SOD and GPX (Verspaget et al., 1991; Mulder et al., 1991). Decreased activities of antioxidant enzymes such as SOD and CAT might be due to the overwhelming effects of free radicals, as evidenced by the elevated levels of lipid peroxidation. Cellular antioxidant enzymes such as SOD, CAT and GPX and free radical scavengers such as GSH protect cells and tissues against noxious free radicals. An imbalance between cellular pro-oxidant and antioxidant levels results in oxidative stress that leads to tissue damage. The antioxidant enzymes react directly with reactive oxygen species (ROS) to yield non-radical products. Superoxide dismutase, a mitochondrial as well as cytosolic enzyme, dismutes O2− to H2O2, which is decomposed by CAT to H2O. Overproduction of these radicals has an inhibitory effect on the enzymes responsible for the removal of ROS such as CAT. It has been reported that superoxide radicals inhibit CAT activity and that H2O2 suppresses SOD activity (Hassan and Fridovich, 1978), which might explain the inhibition of these enzymes after TNBS administration. Therefore, the natural balance between TNBS-induced ROS production and free radical scavengers may be down-regulated, leading to tissue injury (Nieto et al., 2000). Thus, the intestinal mucosa in IBD may be in a constant state of oxidative stress, posing a serious threat to intestinal tissue homeostasis. So increased oxidative stress and impairment of antioxidant defences might contribute to the pathogenesis of IBD. The present study results with the TNBS-induced lipid peroxidation are similar to a study conducted in the recent past (Loquercio et al., 1996). This study showed the oxidative/antioxidative status in the colon after TNBS administration causes a significant decrease in the antioxidant enzyme activities and increased lipid peroxidation.

The effects of natural honey in IBD were studied by the Bilsel et al. (2002) and Mahgoub et al. (2002) acetic acid-induced and TNBS-induced IBD models. The studies concluded that honey is effective in the treatment of IBD. Honey given orally and per rectum showed dose-dependently afforded protection against acetic acid-induced colonic damage. There was almost 100% protection with the highest dose (5 g/kg) (Bilsel et al., 2002). Our study also found similar results in different parameters but the only difference is that Manuka honey was used orally and increasing the doses beyond 5 g/kg did not show more benefits.

Treatment at different doses with Manuka honey obtained from a plant from native New Zealand the manuka tree, Leptospermum scoparium (Myrtaceae), showed a marked improvement and both the doses showed similar efficacy so there was no dose dependent effect of Manuka honey in TNBS-induced IBD. Recent reports suggest the presence of flavonoids in Manuka honey (Weston et al., 1999). Using the TNBS-induced colitis model, a significant enhancement in the activities of antioxidants such as SOD, CAT, GPX and reduced glutathione were observed after treatment with Manuka honey. Of the non-enzymatic antioxidants, reduced glutathione is the most abundant endogenous thiol containing tripeptide present in millimolar concentrations in eukaryotic cells which has been observed.
to be enhanced after Manuka honey treatment. It plays a pivotal role in the maintenance of the balance of cellular reduction and oxidation (redox) reactions and acts as a radical scavenger due to reducto-active sulphhydryl groups directly reacting with oxidants. In addition, GSH is involved in protein and DNA synthesis, amino acid transport, activation of metabolism, catalysis (as a coenzyme) and detoxification of electrophiles either through direct reaction with reactive intermediates or via conjugation reactions catalysed by GST (Meister and Anderson, 1983). Of these actions, protection against oxidative damage caused by ROS is the most important function of GSH. A lowered glutathione content has generally been considered as an ‘index’ of the increased formation of ROS, and glutathione depletion in mammalian cells causes cell damage by oxidative stress (Jain et al., 1991; Martensson et al., 1991). It has been reported that GSH levels are reduced in patients with ROS related diseases (Djordjevic, 2004). A depletion of GSH can lead to increased lipid peroxidation with concomitant changes in membrane permeability and cellular damage, but an increased GSH level enhances the antioxidant potential and cellular functions.

In the present study, the glutathione level increased significantly ($p < 0.05$) after Manuka honey treatment suggesting its protective effect in the colon. The major enzymatic antioxidants include SOD, CAT and GPx, although present in small quantities in the colon compared with the liver. It was found that the SOD and GPx activities peaked and reached their control values after treatment with the Manuka honey compared with CAT. This indicates that these are primary enzymes which play a major role in the detoxification of ROS. Glutathione directly reacts with ROS, whereas GPx catalyses the destruction of hydrogen peroxide and hydroperoxide by utilizing GSH and NADPH (Meister and Anderson, 1983; Grant and Dawes, 1996). It is likely that the increased GPx activities due to Manuka honey might provide a second line of cellular defence in the colon against TNBS-induced oxidative stress.

In conclusion, Manuka honey is efficacious in TNBS-induced IBD and there is no dose dependent effect of Manuka honey, but these results require further confirmation in human studies.

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