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Full Length Research Paper

Potential antioxidant and anti-inflammatory effects of *Hyphaena thebaica* in experimentally induced inflammatory bowel disease

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Ulcerative colitis and Crohn's disease are chronic recurrent inflammatory bowel disease (IBD) of unknown origin. Oxidative stress is believed to be a key factor in the pathogenesis of the mucosal damage in IBD. The aqueous extract of *Hyphaena thebaica* fruits showed an antioxidant activity; this is due to the substantial amount of their water-soluble phenolic contents. This study was undertaken to study the effect of *H. thebaica* in experimentally induced IBD. Sprague Dawley rats were pretreated orally for 5 days with distilled water in control, *H. thebaica* 500 mg/kg in acetic acid + *H. thebaica* group and 5-aminosalisylic acid (5-ASA) 100 mg/kg in acetic acid + 5ASA groups. Colitis was induced by transrectal administration of 4% acetic acid on 5th day. All the animals were sacrificed with ether overdose 48 h after colitis induction, and 10 cm colon segment was resected from distal end. Colon was weighed (for disease activity index) and scored macroscopically and microscopically after histological staining. Biochemical assessments included myeloperoxidase (MPO) and tissue catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD) measurements. *H. thebaica* showed significant prevention of increase in colon weight and disease activity index along with decrease in macroscopic and microscopic lesion score as compared to control group. Significant improvement was observed in the levels of MPO, CAT and SOD, except GSH. However, the effect of *H. thebaica* was significantly less than 5-ASA. *H. thebaica* at 500 mg/kg showed significant amelioration of experimentally induced IBD, which may be attributed to its antioxidant and anti-inflammatory properties.

Key words: Colitis, antioxidant, *Hyphaena thebaica*, myeloperoxidase, catalase, superoxide.

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are two major categories of inflammatory bowel diseases (IBDs). Although the etiology and pathophysiology of IBD still remain unclear, immune dysfunction, reactive oxygen species (ROS), inflammatory mediators and cytokines play important roles in its development and recurrence (Sellin and Pasricha, 2006). It is supposed that homeostasis is disrupted in IBD patients because of over-expression of inflammatory cytokines (TNF-α, IL-1, IL-6) and/or lower expression of regulatory or anti-inflammatory cytokines (IL-2, IL-4, IL-10, TGF-β) (Lih-Brody et al., 1996).

Development of ulcerative colitis is characterized by increased mucosal infiltration by neutrophils and monocytes which, having become activated, release proinflammatory cytokines-tumor necrosis factor α (TNF-

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α), interferon-γ (IFN- γ), interleukin-1β (IL-1β) and free oxygen radicals, induce increased expression of iNOS COX-2, nuclear transcription factor NF-kB, protein p66, and enhancement of the lipoperoxidation processes. These changes cause destructive affections of the mucous membrane of the large intestine, for example impairment of mucous barrier, swelling, ulcers, erosions, and hemorrhages (Hosoi et al., 2001; Martin et al., 2005). The destructive changes the intestinal mucosa is subjected to due to ulcerative colitis are associated with increased numbers of active forms of oxygen, enhanced synthesis of NO, expression of iNOS by epithelial cells, macrophages, and neutrophil infiltraton into the damaged mucous membrane. Concomitantly, the activity of myeloperoxidase, the marker of inflammation was enhanced (Tanaka et al., 2002).

Common drugs that are administrated for the management of IBD include sulfasalazine, 5-ASA derivatives and glucocorticoids. Immunosuppressants, antibiotics and monoclonal antibodies (Infliximab) are also occasionally used for intractable disease conditions (Mcquaid, 2007). These therapeutic agents have side effects and they could not appropriately cure IBD patients (Cross et al., 2008). In many studies, it has been reported that antioxidants show beneficial effects in experimental colitis (Nosal'ova et al., 2000).

Medicinal plants and plant products are the oldest and tried health care products. Their importance is growing not only in developing countries but in many developed countries. *Hyphaene thebaica* (L.) mart is one of the plants used in ethnomedicine and belongs to the family palmae and subfamily Borassoideae. It grows commonly in both Sahel and Sahara regions of Africa (Vonmaydell, 1986). Locally, various extracts and decoction of *H. thebaica* are used in the treatment of bilharzia, haematuria, hypertension and as a haematinic agent (Adaya et al., 1977; Vonmaydell, 1986; Kamis et al., 2003).

Research on the fruit pulp of *H. thebaica* showed that it contains nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid (Kamis et al., 2003). The identification of compounds, by thin-layer chromatography, showed that the fruit contains significant amounts of saponins, coumarins, hydroxycinnamates, essential oils and flavonoids. The fruit also lowers blood pressure in animal models (Sharaf et al., 1972). The aqueous extract of doum fruits showed an antioxidant activity; this is due to the substantial amount of their water-soluble phenolic contents (Hsu et al., 2006). Acetic acid induced colitis model is similar to human ulcerative colitis in terms of histological features, and has been used extensively in many experimental studies of IBD (Nosal'ova et al., 2000; La et al., 2003). The effect of various herbal drugs (but not *H. thebaica*) on experimental models of IBD has been reported earlier with the antioxidant potential as the main mechanism of action against IBD (Ko et al., 2005; Zeytunlu et al., 2004), as the plant *H. thebaica* is thought to possess antiinflammatory and antioxidant properties (Mohamed et al., 2010). This study was undertaken to study the effect of *H. thebaica* in experimentally induced IBD and to find its probable mechanism of action including its antioxidant potential.

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from Sigma chemical company (St Louis, MO, USA).

Plant

The root of *H. thebaica* (L.) mart was obtained from the plant department of faculty of agriculture, Mansoura university.

Preparation of suspension

The root of the plant was treated according to the method of Joslyn (1970). It was dried in an oven for about six hours at 60°C followed by sun drying for days. The dried root was ground into fine powder using mortar and pestle. The powder was sieved through a 0.25 mm sieve. Aqueous suspension was constituted by dissolving 5 g of the powdered root in 100 ml of distilled water and stored at low temperature. The suspension was shaken vigorously to obtain a homogenous mixture before administration.

Animals and treatments

Adult Sprague Dawley rats of both sexes (n = 24) weighing 250 to 300 g were housed individually in a light- and temperature-controlled room on a 12 to 12 h light–dark cycle, in which the temperature (22 ± 2°C) and relative humidity (65 to 70%) were kept constant. The animals were fed a standard pellet lab chow, and food was withdrawn overnight before induction of colitis, but access to water was allowed *ad libitum*. The experiments performed were approved by the Institutional Animal Care and Use Subcommittee of our university. The animals were divided into four groups with six animals in each group as follows:

1. Group A (normal control) – received distilled water 10 mL/kg/day, p.o.
2. Group B (acetic acid) – received distilled water 10 mL/kg/day, p.o.
3. Group C (acetic acid + H.thebaica) – received aqueous suspension of pulp extract of *H. thebaica* 500 mg/kg/day, p.o.(Modu et al., 2000, 2001)
4. Group D (acetic acid + 5ASA) – received 5-ASA 100 mg/kg/day p.o.

The animals were pretreated with the respective drugs (volume of drugs was kept constant at 10 ml/kg) for 5 days, along with the normal diet. On the 5th day, animals were kept fasting for 12 h (overnight) and IBD was induced next morning in Groups B, C and D by administration of 1 ml of 4% acetic acid solution transrectally (TR). Group A (normal control) animals received 0.9% normal saline (TR) instead.

Induction of colitis

For induction of IBD, an 8 mm soft pediatric catheter was advanced 6 cm from the anus under low-dose ether anesthesia. Rats were in Trendelenburg position during this process and 1 ml of 4% acetic acid or 0.9% normal saline solution was slowly administered TR. The rats were maintained in head-down position for 30 s to prevent a leakage, and the rest of the solution was aspirated. After this process, 2 ml of phosphate buffer solution with pH 7 was administered (TR) (Zeytunlu et al., 2004).

Histological analyses

All the animals were sacrificed after 48 h of IBD induction, by ether overdose. Abdomen was opened and colons were exposed. Distal 8 cm of colon was excised and opened by a longitudinal incision. After washing the mucosa with saline solution, mucosal injury was assessed macroscopically using the scale of Morris et al. (1989) as follows: no damage (0); localized hyperemia but no ulceration (1); linear ulcer without significant inflammation (2); linear ulcer with significant inflammation at one site (3); two or more sites of ulceration and inflammation (4) and two or more sites of ulceration and inflammation or one major site of inflammation and ulcer extending > 1 cm along the length of colon (5). Disease activity index (DAI) was measured as the ratio of colon weight to body weight, which was used as a parameter to assess the degree of tissue edema and reflects the severity of colonic inflammation (Ko et al., 2005). Moreover, a 6 to 8 mm sample block of the inflamed colonic tissue with full thickness was excised from a region of grossly visible damage for histological analysis. Formalin fixed tissue samples were embedded in paraffin and stained with Hand E stain. Colonic tissues were scored for histological damage using the criteria of Wallace and Keenan (1999): 0 = intact tissue with no apparent damage; 1 = damage limited to surface epithelium; 2 = focal ulceration limited to mucosa; 3 = focal, transmural inflammation and ulceration; 4 = extensive transmural ulceration and inflammation bordered by normal mucosa; 5 = extensive transmural ulceration and inflammation involving the entire section.

Biochemical assessments

After scoring, the colonic tissue samples were homogenized with 10 volumes of ice-cold 0.25 M sucrose and centrifuged at 14,000 rpm to measure the biochemical parameters in the resulting supernatant.

Measurment of myeloperoxidase (MPO) activity

To measure MPO activity, colonic samples were minced on ice and homogenized in 10 ml of ice-cold 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl trimethyl ammonium bromide (HETAB). The homogenates were then sonicated and centrifuged for 20 min at 12,000 g. MPO activity was measured spectrophotometrically as follows: 0.1 ml of supernatant was combined with 2.9 ml of 50 mM phosphate buffer in 0.0005% H_2O_2. The change in absorbance was measured spectrophotometrically at 460 nm. One unit of MPO activity is defined as the change in absorbance per minute at room temperature, in the final reaction. MPO activity (U/g) = X/weight of the piece of tissue taken, where X = 10 × change in absorbance per minute/volume of supernatant taken in the final reaction (Krawisz et al., 1984).

Assessment of antioxidant status in colonic tissue

Glutathione (GSH) activity

Glutathione (GSH) level was determined according to method of Beutler (1975). The reaction mixture contained supernatant, phosphate buffer and 5,5'-dithio-bis 2-nitrobenzoic acid (DTNB) in a final volume of 10 ml. A blank was also prepared. The absorbance was immediately read spectrophotometrically at 412 nm before and after addition of DTNB. The values were determined from the standard curve.

Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) was measured according to the method of Fridovich (1983). Assay medium consisted of 0.01 M phosphate buffer, 3-cyclohexilamino-1-propanesulfonic acid (CAPS), saturated NaOH with pH 10.2, solution of substrate (0.05 mM xanthine, 0.025 mM *P*-iodonitrotetrazolium violet) and 80 µl xanthine oxidase. Absorbance was read spectrophotometrically at 505 nm. SOD was expressed as U/mg of proteins.

Catalase activity

Catalase was measured by the method of Beers and Sizer (1952). Phosphate buffer (2.5 ml, pH 7.8) was added to supernatant and incubated at 25°C for 30 min. After transferring into the cuvette, the absorbance was measured at 240 nm spectrophotometerically. Hydrogen peroxide (650 µl) was added and change in absorbance was measured for 3 min. Values were expressed as µmol/min/mg of protein.

Statistical analyses

Statistical analyses were done using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered as significant.

RESULTS

Acetic acid administration to the acetic acid group caused significant macroscopic ulcerations and inflammations ($P < 0.05$) in rat colon along with significant mucosal injury (Figure 1A and B) microscopically ($P < 0.05$), when compared to the normal control group (Figure 1 and Table 1) ($P < 0.05$). Also, there was significant derangement of biochemical parameters including tissue levels of MPO, GSH, SOD and catalase ($P < 0.05$), showing oxidative stress due to colon damage and colonic inflammation (Figure 3A and B). Aqueous extract of *H. thebaica* showed significant activity against experimentally induced IBD when compared to the acetic acid group ($P < 0.05$), with near normalization of colon architecture both macroscopically as well as microscopically (Table 1 and Figure 1C). Tissue oxidative stress was reduced with significant improvement in tissue levels of SOD and CAT ($P < 0.05$), showing its antioxidant potential, although there was no significant

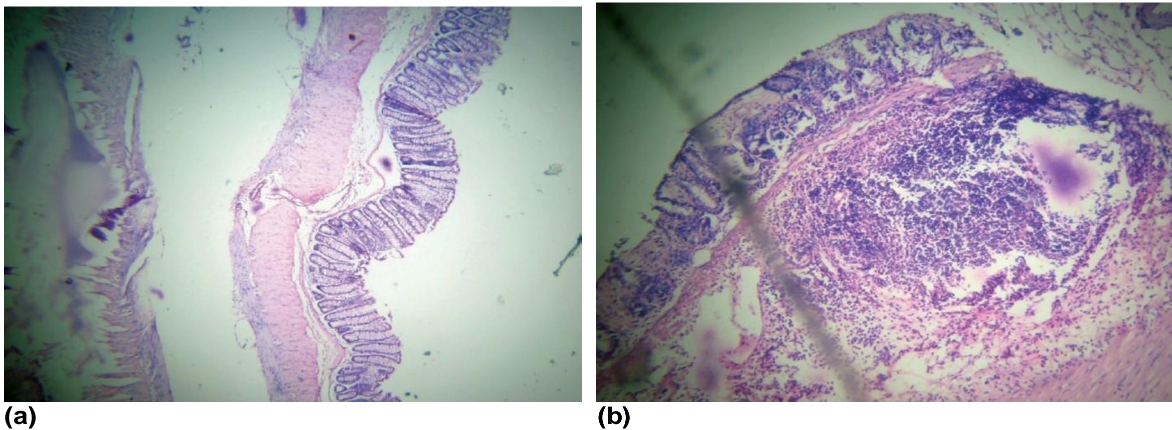

Figure 1. Group A (normal control): Normal mucosal architecture. Group B (experimental control): Extensive necrosis with transmural infiltration.

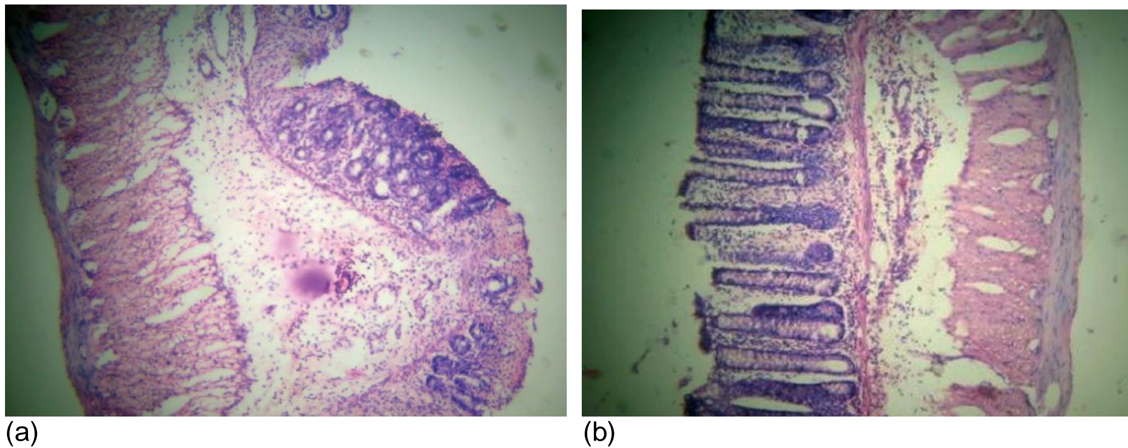

Figure 2. (a) Group C (H. thebaica) Infiltration up to submucosa, architecture maintained. (b) Group D (5-ASA): Near normalization of architecture with mucosal infiltration only.

difference in GSH levels when the two groups were compared ($P > 0.05$). Also, significant improvement in the levels of MPO was observed ($P < 0.05$) (Table 1 and Figures 2A, B, 3A and B).

As for the standard drug 5-ASA, its activity against IBD was significantly better than extract of *H. thebaica* with regard to all the parameters ($P < 0.05$).

DISCUSSION

The results of this study have shown that aqueous extract of *H. thebaica* has got a good potential to suppress experimental colitis in rats, as indicated by macroscopic, microscopic and biochemical evaluations. Acetic acid induced colitis model is similar to human ulcerative colitis in terms of histological features. It affects the distal colon portion and induces non-transmural inflammation, massive necrosis of mucosal and submucosal layers, mucosal edema, neutrophil infiltration of the mucosa and submucosal ulceration. The protonated form of the acid liberates protons within the intracellular space and causes a massive intracellular acidification resulting in massive epithelial damage. The inflammatory response initiated by acetic acid includes activation of cyclooxygenase and lipooxygenase pathways (Sharon and Stenson, 1985; MacPherson and Pfeiffer, 1976).

Phytochemical studies revealed the presence of flavonoids, coumarins and saponins in *H. thebaica.* Various antioxidant flavonoids and coumarin derivatives have been reported as protective products to prevent and treat intestinal inflammatory processes induced by different chemical inductors of experimental colitis (Galvez et al., 2001; Distasi et al., 2004; Luchini et al., 2008; Witaicenis et al., 2010). Several studies have shown that different saponins from *Panax ginseng* and

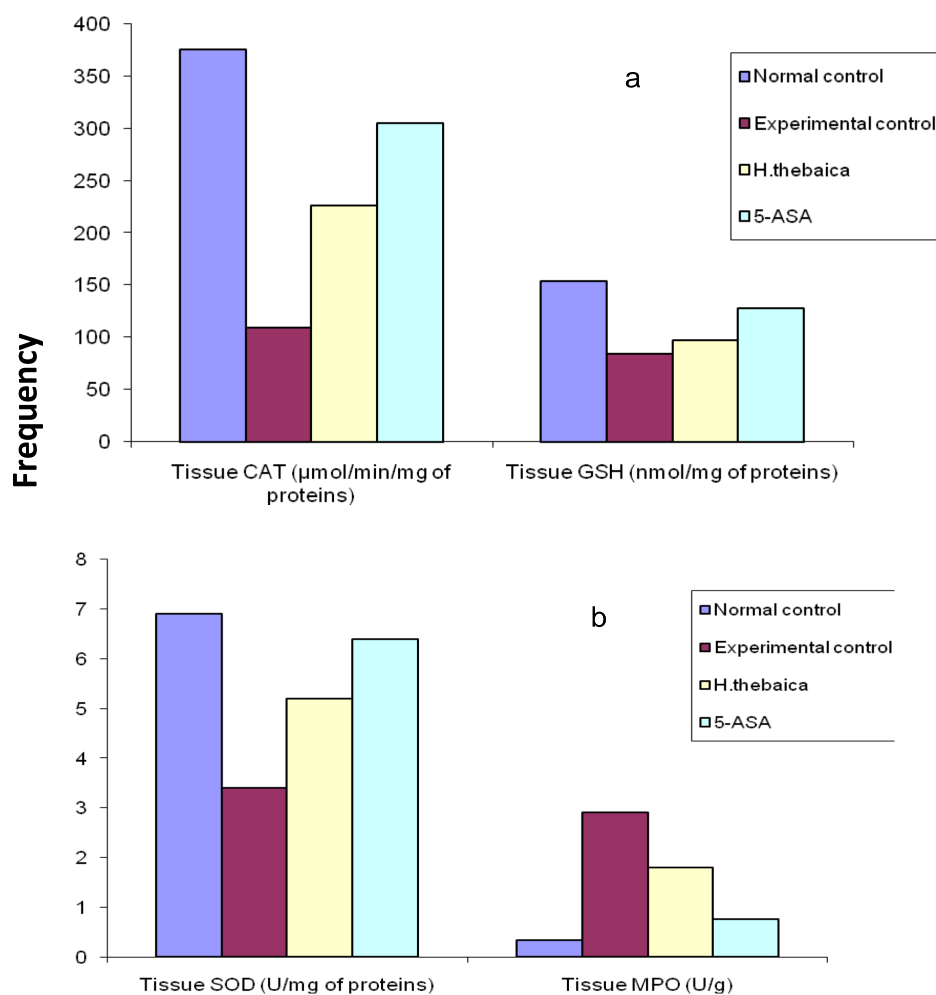

Figure 3. (a) Effect of H. thebaica on tissue CAT (umol/min/mg protein) and GSH (nmol/mg protein) on acetic acid induced colitis in rats. Mean + SE. (6 numbers each). (b) Effect of H. thebaica on tissue SOD (U/mg protein) and MPO (U/g protein) on acetic acid induced colitis in rats. Mean + SE (6 numbers each).

Table 1. Effect Of *H. Thebaica* on macroscopic score, disease activity index and microscopic score (Mean ± SE).

Groups	Microscopic score	Disease activity index (DAI)	Macroscopic score
Normal control	0±0	0.67±0.042	0.33±0.21
Experimental control	4.7±0.21[a]	1.14±0.045[a]	4.67±0.21[a]
H. thebaica	3.0±0.36[a,b]	0.9±0.003[a],[b]	2.0±0.36[a],[b]
5-ASA	1.5±0.22[a,c]	0.76±0.033[a,]	1.0±0.26[a,c]
P<0.05	<0.05	<0.05	<0.05

Value expressed as mean ± SE (number 6). [a]p :in comparsion to normal control[b]p: in comparsion to experimential control. [c]p: in comparsion to standard group.

Codonopsis lanceolata were active compounds in experimental colitis (Joh et al., 2011; Joh and Kim, 2011). Hence, it is plausible that the presence of these classes of natural compounds in the *H. thebaica* contribute to the

observed intestinal anti-inflammatory activity.

The flavonoids found in the extract of *H. thebaica* possess anti-proliferative activity that causes a decrease in the weight and volume of contents of granuloma in inflammation (Koganov et al., 1999). Therefore, this might be its probable mechanism of anti-inflammatory action. MPO, an enzyme found predominantly in the azurophilic granules of neutrophils, is a biochemical marker of neutrophil infiltration, and measurements of its activity have been widely used to detect intestinal inflammatory processes (Yamada et al., 1992; Villegas et al., 2003). Reduction of MPO activity can be interpreted as a manifestation of the anti-inflammatory property of a given compound (Veljaca et al., 1995). The intestinal anti-inflammatory activity of the *H. thebaica* was also related to an inhibitory effect on MPO activity.

Oxidative stress is believed to play a key role in the pathogenesis of IBD-related intestinal damage (Grisham and Granger, 1988). Intestinal mucosal damage in the IBD, including Crohn's disease and ulcerative colitis, is related to both increased free radical production and a low concentration of endogenous antioxidant defense (Koutroubakis et al., 2004). In the present study, the aqueous extract of *H. thebaica* showed an antioxidant activity; this is due to the substantial amount of their water-soluble phenolic contents (Hsu et al., 2006). Furthermore, flavonoids are a large group of naturally-occurring plant phenolic compounds that inhibit lipid oxidation by scavenging radicals or by other mechanisms such as singlet oxygen quenching, metal chelation, and lipoxygenase inhibition (Yanishlieva-Maslarova, 2001; Steenkamp et al., 2013)

As proved by the above study and also as described in literature (Mohamed et al., 2010), the extract of *H. thebaica* possesses significant antioxidant property, proving its role in the management of experimentally induced IBD. Hence, it can be concluded from this study that extract of *H. thebaica* has potent activity against experimentally induced IBD, due to its anti-inflammatory and antioxidant properties. Further investigations for its clinical utility are warranted.

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