

Anti-inflammatory activity, safety and protective effects of *Leptadenia pyrotechnica*, *Haloxyton salicornicum* and *Ochradenus baccatus* in ulcerative colitis

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Received: 18 October 2011, Revised: 8 November 2011 Accepted: 9 November 2011

Abstract

Ethanollic extracts of *Leptadenia pyrotechnica*, *Haloxyton salicornicum* and *Ochradenus baccatus* were evaluated for their antioxidant and anti-inflammatory activities. The aim of the present study is to evaluate the effect of these extracts on the extent and severity of ulcerative colitis (UC) caused by intracolonic administration of acetic acid in rats. The tested plants showed high total phenolic and flavonoid contents. The ethanol extracts of *L. pyrotechnica* (400 mg/kg), *H. salicornicum* (200 and 400 mg/kg) and *O. baccatus* (400 mg/kg) produced significant reduction of carrageenan-induced paw edema. It was noticed that oral pretreatment with the same extracts and doses for 5 days before induction of colitis, protected against diarrhea, colonic ulceration and MPO activity elevation. Results showed a valuable effect of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* extracts against acetic acid-induced ulcerative colitis possibly by their antioxidant and anti-inflammatory properties.

Keywords: *Leptadenia pyrotechnica*, *Haloxyton salicornicum*, *Ochradenus baccatus*, toxicity, anti-inflammatory, ulcerative colitis

Introduction

Leptadenia pyrotechnica (Forsk.)Decne is a typical desert shrub of Asclepiadaceae family growing in different parts of Africa, Asia and Mediterranean region. It is known in the Arabic language as Markh, Assabay and Kalenba (McLaughlin, 2006). It used in folk medici-

ne as an antispasmodic, anti-inflammatory, antihistaminic, antibacterial, diuretic, and expectorant (Cioffi, et al. 2006, Panwara and Tarafdarb, 2006). Modern pharmacological studies have demonstrated that *L. pyrotechnica* extract has analgesic, anti-inflammatory, anabolic, astringent and laxative effects. Chemical studies on *L. pyrotechnica* have shown the presence of bioactive constituents such as steroidal glycosides, cardenolides, alkaloids, flavonoids, triterpenes and polyoxypregnane derivatives (Cioffiet al., 2006). *Haloxylon salicornicum* (Moq.) Bunge ex Boiss. is a desert plant belongs to the family Chenopodiaceae, which has 120 genera and more than 1300 species. In Saudi Arabia, two species are found: *H. persicum* Bunge ex Boiss, Buhse and *H. salicornicum* Bunge ex Boiss (Shaukat, 2000). The plant is reported to be used as anti-diabetic (Ajabnoor et al., 1984), antibacterial (Al-Saeed, 2002) and anti-inflammatory (Al-Shanawani, 1996). *Ochradenus baccatus* (Resedaceae), a yellow green shrub, distributed nearly in all the deserts of Egypt (Tackholm, 1974). New flavonoids quercetin 3-*O*- β -glucosyl (1 \rightarrow 2)- α -rhaminoside-7-*O*- α -rhaminoside and quercetin 3-*O*-*p*-coumaryl (1 \rightarrow 6)- β -glucosyl (1 \rightarrow 6)- β -glucoside-7-*O*- α -rhaminoside, together with known compounds quercetin 3-gentiobioside, isoquercetin, quercitrin and kaempferol were isolated from the aerial part of *O. baccatus* (Barakat et al., 1991).

Ulcerative colitis (UC) is an inflammatory bowel disease that primarily affecting the colonic mucosa. In its most limited form it may be restricted to the distal rectum, while in its most extended form, the entire colon is involved. UC can occur in both sexes and in any age group but most often begins in people between 15 and 30 years of age. In patients with UC, ulcers and inflammation of the inner lining of the colon lead to symptoms of bloody diarrhea, passage of pus, mucus, and abdominal cramping during bowel movements (Baumgart & Sandborn, 2007). The exact causes of UC are still not clear but different factors have been postulated as possible etiologic agents. They are genetic factors, infective agents, immunological disorders, smoking, medications and pathological factors (Berardi, 2000). Currently, there is no an effective therapy to cure the disease but the mainstream treatment depends on reduction of the abnormal inflammation in the colon lining and thereby relieves the symptoms of diarrhea, rectal bleeding, and abdominal pain. Therefore, dexamethasone (DEX), a corticosteroid drug has been mainly used to reduce inflammation and relieve symptoms (Hanauer, Korelitz, Rutgeerts, Peppercorn, Thisted, Cohen & Present, 2004). Nearly 25% of patients with UC requiring steroids therapy become steroid-dependent after one year, and virtually all develop steroid-related adverse events (Faubion et al., 2001). It is therefore necessary to search for alternatives to replace currently used drugs of doubtful efficacy and safety. The aim of the present study is to evaluate antioxidant, safety and anti-inflammatory effect of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* extracts in addition to their potential role in modulating the severity of UC.

Material and methods

Plant Materials

L. pyrotechnica, *H. salicornicum* and *O. baccatus* plants were collected in the wild from Wadi Hagul (Eastern desert of Egypt), during spring season; 2010. The collected plants were identified by Prof. Dr. Ahmed Morsy Ahmed, Plant Ecophysiology, Desert Research Center, Cairo, Egypt. The plants were dried under shade and then grinded to fine powders.

Extraction

The air dried powders (750 g) of each plant were extracted by percolation in 70% aqueous ethanol with occasional shaking for 72 h. The ethanolic extract of each plant was filtered and the residues were re-percolated for three times. The total ethanolic extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* were concentrated under reduced pressure to yield dry extracts of 53, 72, and 58 g, respectively.

Determination of total phenolic and flavonoid contents

Total phenolic content of the tested extracts was carried out according to the Folin-Ciocalteu method (Singleton et al., 1999), using gallic acid as standard. The extracts were oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. Total phenolic content was expressed as gallic acid equivalents (GAE) per mg of extract. Total flavonoid content was determined by a colorimetric method of (Zhisen et al., 1999) and calculated using a quercetin calibration curve. The results were expressed as quercetin equivalents (QE) per mg of extract.

Antioxidant activity

Free radical scavenging activity of the ethanolic extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* was determined with the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay using Trolox (2.5 mM in methanol) as a reference substance (Liu, Li et al., 2002). The results (Mean \pm SD of at least three measurements) were expressed as Trolox equivalent antioxidant capacity (TEAC).

$$\% \text{ inhibition of DPPH activity} = \left(\frac{A-B}{A} \right) \times 100$$

Where A is the optical density of the blank and B is the optical density of the sample.

Biological activities

Animals

Male Wistar albino rats weighing 180–200 g were used for the study. Animals were maintained under standard conditions of temperature (22 \pm 1 °C), relative humidity (55 \pm 10%), and 12-h light: 12-h dark cycle, and fed a standard pellet diet with water *ad libitum*. They were housed in standard polypropylene cages with wire mesh top. All studies were carried out using six animals in each group. The care and handling of the animals were in accordance with the internationally accepted standard guidelines. All animal procedures were approved by an institutional review board of Pharmacy College, Salman Bin Abdulaziz University, KSA.

Preparation of the extracts for biological studies

The total ethanol extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* and the standard; DEX were suspended separately in 3% v/v Tween 80 (vehicle).

Acute toxicity and determination of median lethal dose (LD₅₀)

LD₅₀ of the ethanol extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* were determined in rats according to the method of (Lorke, 1983). Male Wistar albino rats in groups of six, received one of 1000, 2000, or 4000 mg/kg of the tested extracts. Control animals were received the vehicle and kept under the same conditions. Signs of acute toxicity and number of deaths per dose within 24 h were recorded and the LD₅₀ was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no lethality at all.

Doses

The dose selection for the ethanol extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* was based on our preliminary toxicity study in normal rats, which no toxicity was observed with their oral administration in doses up to 4000 mg/kg. Accordingly, experimental doses of 200 and 400 mg/kg that equal to $1/20$ and $1/10$ of the maximum possible dose of the extracts that didn't cause mortalities in rats were selected to be given orally. The reference drug; DEX was given orally at a dose of 0.2 mg/kg. This dose was calculated by converting the therapeutic dose that used in human to rat's dose according to the Table of Paget and Barnes (1964). DEX was chosen as a positive control because it is widely used for treatment of colitis in the clinic. Acetic acid was diluted in saline to be 4% and infused into the colon of rats through a polyethylene catheter at the dose of 5 mL/kg.

Sub-chronic Toxicity

Forty two male Wistar albino rats were randomly divided into 7 equal groups. Rats of the 1st group were given the vehicle in a dose of 5 mL/kg and left as normal control. Rats of the 2nd and 3rd groups were medicated with the ethanol extract of *L. pyrotechnica* in doses of 200 and 400 mg/kg, respectively. Rats of the 4th and 5th groups were administered 200 and 400 mg/kg of the ethanol extract of *H. salicornicum*, respectively. The ethanol extract of *O. baccatus* was given to the 6th and 7th groups in doses of 200 and 400 mg/kg, respectively. All medications were administered orally via the aid of an orogastric cannula for 35 consecutive days. Animals were maintained under identical conditions with food and water *ad libitum* for the entire period with close observation. At the end of the experimental period, blood samples (2 mL) were drawn by puncturing retro-orbital venous sinus of each rat (under ether anesthesia) and centrifuged at 10,000 rpm for 5 minutes. Sera were separated to be used for the biochemical estimations.

Measurement of liver and kidney function markers

Liver functions were evaluated by measuring the serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) following the method of (Reitman and Frankel, 1957). Serum levels of total bilirubin (Walter & Gerarde, 1970), total proteins (Henary et al., 1974) and albumin (Doumas et al., 1971) were also assayed. Serum concentrations of urea (Wills & Savory, 1981) and creatinine (Kroll, Roach, Poe & Elin, 1987) were determined colorimetrically as measures of kidney functions.

Anti-inflammatory activity

The anti-inflammatory activity was evaluated in male Wistar albino rats using a carrageenan-induced paw edema test according to the method of Winter et al. (1962). Eight groups of rats each consisting of 6 animals were fasted overnight before the experiment with free access to water. Rats of the 1st (normal control) and 2nd (reference) groups were treated orally with the vehicle (5 mL/kg) and DEX (0.2 mg/kg), respectively. Animals of the 3rd and 4th groups were orally given the ethanolic extracts of *L. pyrotechnica* (200 and 400 mg/kg, respectively). Rats of the 5th and 6th groups were medicated orally with the ethanol extract of *H. salicornicum* (200 and 400 mg/kg, respectively). The ethanol extract of *O. baccatus* was administered orally to the 7th and 8th groups in doses of 200 and 400 mg/kg, respectively. After 30 min, acute inflammation was induced by subplantar injection of 0.1 mL of carrageenan (1% in saline) in the left hind paw of all rats. The paw volumes up to the tibiotarsal joint were measured in mL using a plethysmometer immediately before and 3 h following carrageenan injection. The mean increases in injected paw edema with respect to initial paw volume were calculated and the percentage inhibition of paw edema with respect to control group was calculated using the formula:

$$\% \text{ inhibition of paw edema} = 1 - \left(\frac{V_t}{V_c} \right) \times 100$$

Where: V_t and V_c are the mean change in paw volume of treated and control rats, respectively.

Effect on ulcerative colitis

Fifty four male Wistar albino rats were divided into 9 equal groups: Groups 1 and 2 (normal and colitis control groups, respectively) were given the vehicle in a dose of 5 mL/kg. Group 3 (reference group) was given DEX in a dose of 0.2 mg/kg. Groups 4 and 5 were administered the ethanol extract of *L. pyrotechnica* in doses of 200 and 400 mg/kg, respectively. Rats of groups 6 and 7 were medicated with the ethanol extract of *H. salicornicum* (200 and 400 mg/kg, respectively). Groups 8 and 9 were treated with *O. baccatus* extract in doses of 200 and 400 mg/kg, respectively. All medications were administered orally via the aid of an orogastric cannula, once a day for 5 consecutive days and the last dose was administered 2 h before colitis induction.

Induction of ulcerative colitis

Rats were fasted for 24 h with access to water *ad libitum* after which they were lightly anesthetized with ether. A polyethylene catheter with 2 mm diameter was inserted through the rectum into the colon to a distance of 8 cm (Ghaneya and Soliman, 2010). For Ulcerative colitis induction, 2 mL of 4% (v/v) acetic acid was infused into the colon of all rats (except the normal control group) through the catheter, held in place for 30 sec, and then flushed with 5 mL of phosphate buffer solution; pH=7. The catheter was left in place for few seconds then gently removed.

Assessment of colonic lesions

Two days after the induction of colitis, each rat was inspected and diarrhea was recorded. Rats were sacrificed using ether anesthesia and colonic segments (8 cm in length a-

Table 1. Total Phenolics, Total flavonoids and antioxidant activity of the tested plant extracts.

Plant	Total phenolics (mg GAE per g extract)	Total flavonoids (mg QE per g extract)	Antioxidant activity	
			TEAC	%
<i>L. pyrotechnica</i>	158.3 ±6.25	89.0±3.40	1.84±0.07	50
<i>H. salicornicum</i>	164.4±7.22	92.0±2.55	1.78±0.10	46
<i>O. baccatus</i>	145.3±6.40	85.0±3.11	1.48±0.08	37

GAE = gallic acid equivalent, QE = quercetin equivalent, TEAC = Trolox equivalent antioxidant capacity.

nd 3 cm proximal to the anus) were excised, opened along its mesenteric border, and rinsed thoroughly in ice-cold normal saline. The colon specimen of each rat was weighted and wet weight/length ratio was calculated as ratio of the colon specimen weight vs its length (g/cm). It was used as a parameter to assess the degree of colon edema, which reflected the severity of colitis.

The specimens were examined under a dissecting microscope and all visible damages were evaluated using the scoring system reported by (Morris, et al., 1989) with some modifications. The lesion scores were: 0 = no damage, 1 = Local edema and inflammation without ulcers; 2 = One ulcer without inflammation; 3 = one to two ulcers with inflammation and lesion diameter < 1 cm; 4 = More than two ulcers with lesion diameter 1-2 cm; 5 = Sever ulceration with lesion diameter >2 cm. Ulcer area was measured for each specimen using a 1-mm² grid. Ulcer index was measured by summing the lesion score and the ulcer area for each colon specimen (Minaiyan et al., 2006).

Effect on the activity of myeloperoxidase (MPO) in the colonic mucosa of rats

The colonic mucosa was carefully scraped off from the colon of each rat with a glass slide and snap-frozen in liquid nitrogen, and stored at -80 °C for the assay of MPO activity within a week. The frozen colonic mucosa was homogenized with a homogenizer in phosphate buffered saline then MPO activity was determined by a modified method described by Krawisz et al., (1984).

Statistical Analysis

All the values were expressed as mean±S.E.M. Statistical analysis was done by using SPSS 10 and statistical significance of differences between two means was assessed by unpaired Student's 't' test. Differences at P≤ 0.05 were considered statistically significant.

Results

Determination of total phenolic and flavonoid contents

The present results (Table 1) showed that the ethanol extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* have high total phenolic content (158.3±6.25, 164.4±7.22 and 145.3±6.40 mg GAE per g extract, respectively). *H. salicornicum* and *L. pyrotechnica* were found to have the highest total flavonoid content (92.0±2.55 and 89.0±3.40 mg QE per g ext-

Table 2. Effect of prolonged oral administration of the tested plant extracts for 35 consecutive days on the serum activity of ALT and AST and serum levels of total bilirubin, total protein, and albumin in rats, (n = 6).

Groups	Dose (mg/kg)	ALT (U/L)	AST (U/L)	Total bilirubin (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
Control	00	68.84±2.74	141.45±4.27	1.71±0.11	8.8±0.26	3.9±0.15
	200	60.46±3.15	140.46±5.33	1.76±0.09	8.5±0.22	3.6±0.12
	400	63.52±2.24	138.01±4.61	1.85±0.10	8.4±0.25	3.5±0.14
<i>L. pyrotechnica</i>	200	62.00±4.53	142.62±5.24	1.72±0.12	8.6±0.30	3.7±0.12
	400	64.75±2.40	140.54±4.60	1.62±0.13	8.5±0.22	3.4±0.10
<i>H. salicornicum</i>	200	61.22±3.75	144.61±5.25	1.70±0.10	8.5±0.28	3.5±0.16
	400	67.64±2.55	141.24±4.85	1.76±0.11	8.3±0.27	3.2±0.14
<i>O. baccatus</i>	200					
	400					

ract, respectively) while *O. baccatus* displayed lower total flavonoid content (85.0±3.11 mg QE per g extract).

Antioxidant activity

In the present study *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* extracts showed antioxidant and DPPH radical scavenging activities (Table 1). The highest antioxidant activity was shown by the ethanolic extract of *L. pyrotechnica* (1.84 TEAC). The antioxidant activity of *H. salicornicum* (1.78 TEAC) and *O. baccatus* (1.48 TEAC) extracts are relatively lower than *L. pyrotechnica* extract.

Acute toxicity and determination of LD₅₀

The obtained results indicated that different doses of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* extracts (1000, 2000 and 4000 mg/kg b.wt.) did not produce any symptom of acute toxicity and none of the rats died during 24 h of observation. All rats did not exhibit diarrhea, haematuria, restlessness, uncoordinated muscle movements, and respiratory distress

Table 3: Anti-inflammatory effect of DEX and the tested plant extracts against carrageenan-induced paw edema in rats. (n = 6).

Groups	Dose (mg/kg)	Mean increase in paw volume (mL)	Reduction of paw swelling (%)
Control	00	2.17 ± 0.29	00.00
DEX	0.2	0.75 ± 0.09***	65.43
<i>L. pyrotechnica</i>	200	1.64 ± 0.17	24.42
	400	1.07 ± 0.14**	50.69
<i>H. salicornicum</i>	200	1.46± 0.13*	32.71
	400	1.00± 0.16**	53.92
<i>O. baccatus</i>	200	1.75 ± 0.18	19.35
	400	1.28 ± 0.15*	41.01

Significant at * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

accordingly, it suggested that oral LD₅₀ of the tested extracts were higher than 4000 mg/kg b.wt.

Sub-chronic toxicity

In the present study, oral dosing of the tested extracts to rats in doses of 200 and 400 mg/kg for 35 days did not show any significant effect on the levels of ALT, AST, total bilirubin, total proteins, and albumin in their sera as compared to control (Table 2). No significant change in the mean values of urea and creatinine was estimated in sera of rats following 35 days of extracts administration at doses of 200 and 400 mg/kg when compared with the control (data not shown).

Anti-inflammatory activity

After 3h of carrageenan injection, DEX (0.2 mg/kg) and the ethanolic extracts of *L. pyrotechnica* (400 mg/kg), *H. salicornicum* (200 and 400 mg/kg) and *O. baccatus* (400 mg/kg) significantly reduced the mean increase of paw volume as compared to the initial paw volume (Table 3). The percentages of reduction of paw swelling in case of 400 mg/kg of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* extracts after 3h of carrageenan injection were 50.60%, 53.92% and 41.01%, respectively corresponding to 65.43% in DEX group. *H. salicornicum* at 200 mg/kg reduced the paw swelling by 32.71 %. The better anti-inflammatory effect was recorded with 400 mg/kg of *H. salicornicum* and *L. pyrotechnica* but is less pronounced than that of DEX.

Anti-ulcerogenic effect

In the present study, no abnormal changes were observed in rats of the normal control group suggesting that handling procedure had no interference with the experimental outputs. Experimental colitis was accompanied by marked anorexia, prostration, hypomotility and piloerection after acetic acid challenge (data not shown). Diarrhea, as evidenced indirectly by perianal fur soiling, was prominent among colitic animals. The incidence of diarrhea, lesion score, ulcer area and ulcer index were used as the indicators for the effectiveness of the tested extracts against colitis induced by acetic acid in rats. Rats of the control colitis group developed severe diarrhea (100 %) after rectal acetic acid infusion. DEX and the ethanolic extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* at the high dose (400 mg/kg) reduced the incidence of diarrhea to 16.7, 33.3, 33.3 and 50%, respectively and the diarrhea was much milder. The low dose of the tested extracts (200 mg/kg) had weak effect in protecting against diarrhea of the rats with colitis except *H. salicornicum* that had a moderate protective effect (66.7%).

The intestinal damage that induced by acetic acid was associated with a significant increase of wet weight/length ratio of the colon specimens as an indicator of inflammation. The wet weight/length ratio increased 3-fold in rats with acetic acid colitis compared to normal rats (0.96 ± 1.73 vs 0.30 ± 0.13 g/cm). This ratio was greatly improved in rats that pre-medicated with 0.2 mg/kg of DEX (0.35 ± 0.05 g/cm) and 400 mg/kg of the ethanol extracts of *L. pyrotechnica* (0.47 ± 0.07 g/cm), *H. salicornicum* (0.43 ± 0.05 g/cm) and *O.*

Table 4. Effects of DEX and the tested plant extracts on the macroscopic parameters of ulcerative colitis induced by acetic acid in rats. (n = 6).

Groups	Dose (mg/kg)	Diarrhea (%incidence)	Lesion score (0-5)	Ulcer area (cm ²)	Ulcer index	Wet W/L ratio (g/cm)
Normal control	00	00.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.30 ± 0.04
Colitis control	00	100.0	4.6 ± 0.24	5.3 ± 0.39	9.9 ± 0.55	0.96 ± 0.11
DEX	0.2	16.7	2.1± 0.15***	2.2± 0.18***	4.3± 0.25***	0.35± 0.05***
<i>L. pyrotechnica</i>	200	83.3	4.0 ± 0.22	4.7 ± 0.28	8.7 ± 0.45	0.80 ± 0.12
	400	33.3	2.9± 0.19***	3.5± 0.24**	6.4± 0.42**	0.47± 0.07**
<i>H. salicornicum</i>	200	66.7	3.7 ± 0.25*	4.3 ± 0.20*	8.1 ± 0.41*	0.72 ± 0.06
	400	33.3	2.8± 0.18***	3.2± 0.25**	6.0± 0.38**	0.43± 0.05**
<i>O. baccatus</i>	200	83.3	4.2 ± 0.23	5.0 ± 0.29	9.2 ± 0.43	0.88 ± 0.07
	400	50.0	3.3± 0.21**	3.9 ± 0.28*	7.2 ± 0.40*	0.56± 0.07*

Significant at * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

baccatus (0.56±0.07 g/cm). Morphologically, a thickened wall and brown to black lesions were observed in the injured colon specimens of control colitis rats, 2 days after rectal infusion of acetic acid. Under dissecting microscope, hyperemia, edema, erosion, and sever ulceration were also observed. Control colitis rats showed lesion score, ulcer area and ulcer index values of 4.6 ± 0.24, 5.3 ± 0.39 cm² and 8.9 ± 0.55, respectively (Table 4).

These inflammatory indices were significantly improved by oral dosing of DEX and the ethanol extracts of *L. pyrotechnica* (400 mg/kg), *H. salicornicum* (200 and 400 mg/kg) and *O. baccatus* (400 mg/kg) for 5 days prior to ulcer induction. The 200 mg/kg dose level of the ethanol extracts of *L. pyrotechnica* and *O. baccatus* failed to improve the lesion scores. The anti-ulcerative effect of the tested extracts was lower than that of DEX. On the other hand, *O. baccatus* had a lower protective efficacy.

Effect on the activity of myeloperoxidase (MPO) in the colonic mucosa of rats

MPO activity indicates the degree of inflammatory cell infiltration, which is a marker of acute inflammation. In this study, the activity of MPO was assessed in the colonic mucosa of all rats and the results are shown in Figure 1. The myeloperoxidase assay indicates that the activity of MPO was very low in the normal control rats (0.14 ± 0.03 U/g).

The activity of MPO severely elevated (more than 80-fold) in rats with acetic acid colitis (11.38 ± 1.60 U/g). The ethanol extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* at the dose of 400 mg/kg significantly inhibited the elevation of MPO activity in the rats with colitis. The activity was decreased to 7.26 ± 0.71; 5.47± 0.55 and 8.26 ± 0.78 U/g, respectively. The effect of DEX in inhibiting the activity of MPO (4.50 ± 0.47 U/g) was slightly stronger than *H. salicornicum* extract.

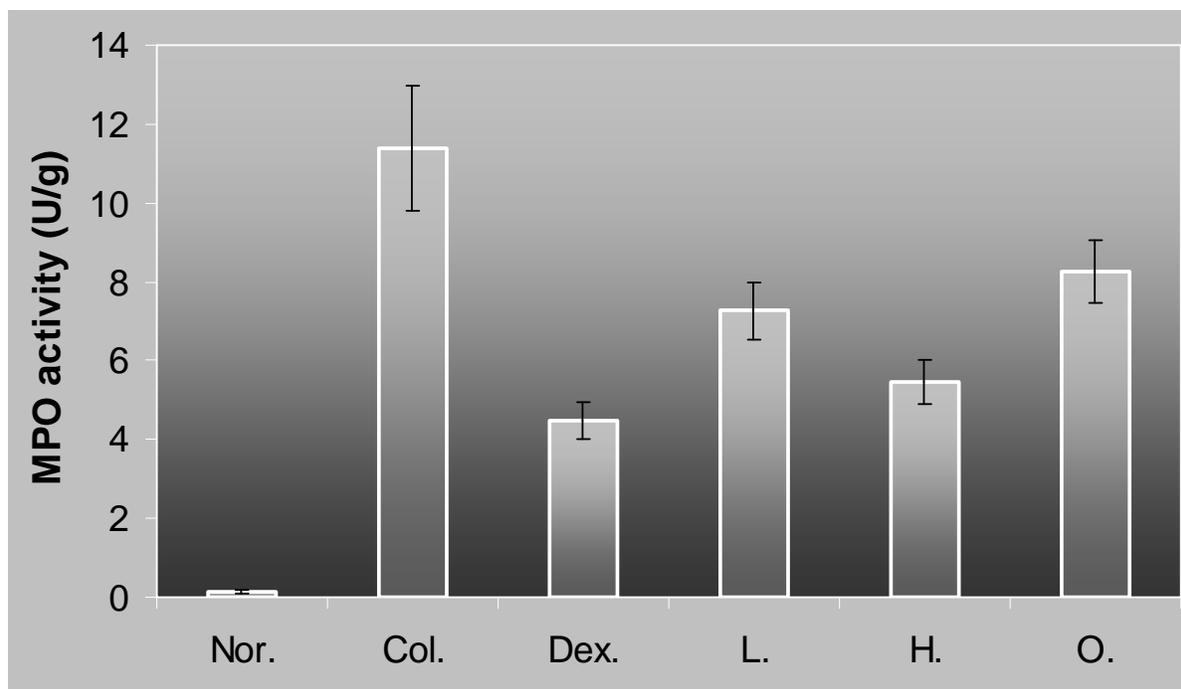


Figure 1. The effect of DEX (0.2 mg/kg) and the ethanolic extract (400 mg/kg) of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* on MPO activity of colonic mucosa in rats with colitis induced by acetic acid. (Nor. = Normal control, Col. = colitis control, Dex.= dexamethasone, L = *L. pyrotechnica*, H = *H. salicornicum*, O = *O. baccatus*).

Discussion

Ethanolic extracts were tested phytochemically to ensure the presence of polyphenols and flavonoids. Our results showed that the ethanol extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* have high total phenolic and flavonoid contents. Phenolic compounds are secondary metabolites which synthesize in plants. They possess some biological properties such as: antioxidant, anti-apoptosis, anti-aging, and anti-inflammation. Flavonoids are a large group of ubiquitous molecules synthesized by plants. Many studies have shown that flavonoids play important pharmacological roles against various human diseases, such as cardiovascular diseases, cancer, inflammation and allergies (Vinson et al., 1998). In a previous study, some phenolic compounds, flavonoids, alkaloids and triterpenoids were isolated from the aerial parts of *L. pyrotechnica* (Cioffi et al., 2006).

The model DPPH provides a method to evaluate antioxidant activity in a relatively short time compared to the other methods. The antioxidant activity of the tested plants could be attributed to their total phenolic and/or flavonoidal contents. There is a highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups (Vinson et al., 1998). It was also reported that phenolic compounds are effective hydrogen donors, making them very good antioxidants (Yen, Duh, & Tsai, 1993). In addition, flavonoids act as scavengers of various oxidizing species i.e. super oxide anion (O_2^-), hydroxyl radical or peroxy radicals, they also act as quenchers of singlet oxygen (Das and Ratty, 1986).

All rats treated with different doses of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* extracts were alive during 24 h of observation. It suggested that the LD₅₀ of these extracts was higher than 4000 mg/kg. Therefore, the tested plants can be categorized as highly safe since substances possessing LD₅₀ higher than 50 mg/kg are non-toxic (Buck et al., 1976). The non-toxic nature of the ethanolic extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* in acute toxicity study is well supported by the normal levels of biochemical data (ALT, AST, total bilirubin, total proteins and albumin) following 35-days treatment period in rats. The serum transaminase level is most widely used as a measure of hepatic injury, due to its ease of measurement and high degree of sensitivity. It is useful for the detection of early damage of hepatic tissue. Since the activity of ALT and AST are specific assayable liver enzymes, their normal levels in serum of experimental groups of rats treated for 35 days means that the three tested plants are not hepatotoxic. Urea and creatinine are the most sensitive biochemical markers employed in the diagnosis of renal damage. In kidney damage, there will be retention of urea and creatinine in the blood (Nwanjo et al., 2005), therefore marked increase in serum urea and creatinine are indications of functional damage to the kidney (Panda, 1999). By these indicators, ethanol extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* are therefore, not nephrotoxic in rats.

The ethanolic extracts of *L. pyrotechnica* (400 mg/kg), *H. salicornicum* (200 and 400 mg/kg) and *O. baccatus* (400 mg/kg) showed an anti-edematogenic effect on paw edema after 3h of carrageenan injection. Carrageenan-induced inflammation in the rat paw represents a classical model of acute inflammation that used for evaluation of anti-inflammatory activity of drugs or plant extracts. In this respect, it was reported that phenolic compounds might act as anti-inflammatory agents (Sosa et al., 2005). Moreover, inhibition of leucocyte chemotaxis may cause the anti-inflammatory effect of these compounds. Another suggested mechanism of anti-inflammatory action of phenolic compounds is the prevention of the production of oxygen free radicals by leucocytes (Azuma et al., 1986). Similarly, flavonoids are known to have an anti-inflammatory property (Kim, Son, Chang, Kang, 2004). Accordingly, we presume that the presence of phenols and/or flavonoids in the tested extracts may be contributing to their anti-inflammatory activity in rats.

The model of acetic acid induced colitis shares many of the histological features of UC in human beings including mucosal edema and submucosal ulceration (Sharon and Stenson, 1985). The protective effect of the tested extracts against acetic acid induced ulcers could be attributed to their phenolic and/or flavonoid content and their reactive oxygen species scavenging property. The antioxidative mechanism of the tested extracts against colon mucosal lesions was supported by their *in vitro* antioxidant potency.

MPO was found predominantly in neutrophils, monocytes, and macrophages (Sugiyama et al., 2001) and has been implicated as a participant in tissue injury during inflammatory diseases (Zhang et al., 2002). Several reports have demonstrated increased neutrophil infiltration in inflammatory mucosa (Otamiri et al., 1988). Such infiltration might be regarded as a trigger of free radicals release. Increased production of free radicals and impaired antioxidant defense mechanisms are postulated to be causative factors in inflammatory diseases (Han and Meydani, 2000). Accordingly, estimate-on of MPO activity in colonic mucosa has been used as an indicator of neutrophil influx into inflamed colon tissue (Boughton-Smith, Wallace, Morris & Whittle, 1988). In the present investigation, the ethanol extracts of *L. pyrotechnica*,

H. salicornicum and *O. baccatus* at the dose of 400 mg/kg attenuated colon mucosal damage and subsequently reduced MPO activity in colonic tissues. This protective effect may be attributed to the ability of the tested extracts to reduce neutrophil infiltration in inflamed colonic tissue.

In conclusion, the tested ethanol extracts were well tolerated following acute and sub-chronic treatment and they neither produced overt signs of clinical toxicity nor any signs of hepatotoxicity or nephrotoxicity. Moreover, the ethanol extracts attenuated the macroscopic colonic damage -induced by acetic acid and inhibited the elevation of MPO activity in the colonic mucosa in rats with colitis. These results suggest that the ethanol extracts of *L. pyrotechnica*, *H. salicornicum* and *O. baccatus* may be effective in the prevention of UC through their anti-inflammatory and scavenging effect on oxygen-derived free radicals. However, more detailed phytochemical studies are necessary to identify the active principles and exact mechanism of action.

Acknowledgment

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for the work through the research group project NO RGP-VPP-060.

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