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Research Article
Antiulcer and Anti-ulcerative Colitis Activities of *Haplophyllum tuberculatum* (Forsskal)

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Abstract

Background and Objective: Recently, research has been focused on natural products in the discovery of new anti-ulcerogenic drugs and anti-ulcerative colitis. Family Rutaceae is one of the most important families with many biological investigations. *Haplophyllum tuberculatum* (Forsskal) was used in folk medicine in the treatment of many diseases, especially gastric disorder with no scientific proof. The aim of this study was to investigate and prove their biological activities in the treatment of peptic ulcer and ulcerative colitis. **Materials and Methods:** Extraction of the plant was carried out by using alcohol (95%). Anti-ulcerogenic activity was evaluated using absolute ethanol-induced ulcer model in rats using reference standard drug ranitidine (100 mg kg^{-1}). While the ulcerative colitis was evaluated using the acetic acid-induced model in rats using reference standard drug Dexamethasone (0.1 mg kg^{-1}). Statistical analysis was done by using SPSS 10. The statistical significance of the differences between two means were assessed by unpaired Student's' test. Differences at $p<0.05$, 0.01 and 0.001 were considered statistically significant. **Results:** The total alcohol extract (400 mg kg^{-1}) showed an anti-ulcerogenic activity with different potentials. The total alcohol extract was safe up to 4000 mg kg^{-1} and showed some side effects on liver and kidney functions when administrated orally for 15 consecutive days at a dose of 400 mg kg^{-1} in normal, controlled peptic ulcer and ulcerative colitis. **Conclusion:** Plant extract of *Haplophyllum tuberculatum* showed both potential antiulcer and anti-ulcerative colitis activities. The plant showed curative effect on peptic ulcer with dose dependent (28 and 38% for both 200 and 400 mg kg^{-1}, respectively). In case of treatments of ulcerative colitis the curative effect was also dose dependent (31.51 and 42.74% for both 200 and 400 mg kg^{-1}, respectively) in addition to their use as dietary supplements for Minerals (Sodium, Potassium and Magnesium).

Key words: Anti-ulcerogenic, sub-chronic toxicity, antiulcer activity, ulcerative colitis activity, mineral deficiency

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Peptic Ulcer disease (PU) and Ulcerative Colitis (UC) are the most common types of gastrointestinal tract ulcers which are distributed all over the world. Several types of drugs are available for the treatment of these disorders such as aminosalicylates, Antacids and other synthetics[1]. In most cases, the incidence of relapses and adverse reactions are synthetic antiulcer therapy.

The genus *Haplophyllum* along with about 150 genera belongs to the Family ofthe Rutaceae[1]. The family comprises of 900 species and is distributed in both the temperate and tropical regions in Africa and Australia[1]. The genus *Haplophyllum* is one of the largest Rutaceae including about 70 species. It has a wide geographical distribution from the Mediterranean area to Eastern Siberia[2]. It is distributed from Morocco and Spain in the West to China in the East and from Romania in the North to Somalia in the South[3].

The *Haplophyllum tuberculatum* is well known for its richness in alkaloids, fixed oils, volatile oils and furanocoumarins[4,5]. The ethanol extract of *H. tuberculatum* aerial parts are rich in phenolic compounds[6]. The essential oil of *H. tuberculatum* contains about 30 compounds constituting 99.7% of the total oil[7]. The highest oil components are beta-phellandrene (23.3%), limonene (12.6%), (Z)-beta-ocimene (12.3%), beta-caryophyllene (11.6%), myrcene (11.3%) and alpha-phellandrene (10.9%)[7].

Members of the Rutaceae family have a great economic interest, including wood, food, cosmetic and medicinal uses[8]. *H. tuberculatum* is used to treat malaria, rheumatoid arthritis and gynecological disorders in Saudi Arabia[9]. The flowering aerial parts are used as a decoction for rheumatic pains in Egypt[10]. The leaves are used for relieving arthritis and also used for the treatment of skin infections in Oman[11]. The herb is used as an antispasmodic to treat allergic rhinitis and gynecological disorders, asthma and breathing difficulties in Sudan[12]. The species of genus *Haplophyllum* are used also to treat sterility, constipation, intestinal colic, malaria and against biting insects and flies[1,13].

Moreover, Ethanolic extract of the aerial parts of *H. tuberculatum* showed an efficient antifungal activity against *Aspergillus fumigates*, *Geotricum candidum*, *Syncephalas trumracemosum*, *Curvularia lunata* and *Fusarium oxysporium* and antibacterial activity against *Escherichia coli*, *Salmonella choleraesuis* and *Bacillus subtilis*[7,14]. The volatile parts of the aerial demonstrated a significant antibacterial effect against the *Enterococcus faecalis* and *Lactobacillus acidophilus*[14]. The essential oils from the aerial parts and flowers exhibited a remarkable acute anti-inflammatory activity against carrageenan induced edema in rats, which were found to be comparable to the standard drug at the selected dose[14]. The ethanol extract of the aerial parts exhibited a significant antioxidant activity (98%) as compared to vitamin E[14].

According to the previous information and the use of *H. tuberculatum* in folklore medicine to treat gastro intestinal diorders[13], the plant has been-selected to introduce a new natural product with good antiulcer and anti-ulcerative colitis activities and the research will focus on studding the effect of *H. tuberculatum*.

MATERIALS AND METHODS

Phytochemical part

Plant materials: The aerial part of *H. tuberculatum* (Forsskal) was collected during flowering stage in 2015 from Al-Yamamah territory (Al-Kharj. South of Riyadh. KSA). Three Plant Samples were identified by Dr. Jacob T. Pandalayil (Assistant Professor of Plant Taxonomy, Botany and Microbiology). Plant materials were air-dried in the shade, reduced to fine powder, refilled in tightly closed containers and stored for photochemical and pharmacological studies.

Extraction: The air dried powders (250 g) of *H. tuberculatum* were extracted by percolation in 95% aqueous ethanol (analytical grade), with an occasional shaking for 72 h. The ethanol extracts were filtered and the residues were re-percolated three times. The combined filtrates were concentrated under reduced pressure at low temperature to yield 85 g (total alcohol extract).

Preliminary phytochemical screening: For the determination of the active constituents, the air-dried powder of the investigated plant (*H. tuberculatum*) was subjected to a preliminary phytochemical screening according to the published methods[15,16].

Pharmacological activities

Animals: Swiss albino mice of both sex (26-30 g) and male Wistar rats (180-200 g) were purchased from the animal house of King Saud University, KSA. Animals were housed in standard polypropylene cages with wire mesh top and maintained under standard conditions (temperature 23±1.0°C, humidity 55±10%, 12 h light/12 h dark cycle). They were fed a standard

pellet diet with water *ad libitum* and allowed to adapt to the laboratory environment for one week before experimentation.

Preparation of the extracts for biological studies: The dried plant extract was freshly suspended in distilled water just before administration with the aid of Tween 80.

Acute toxicity (LD_{50}) test: The oral median lethal dose (LD_{50}) of the alcohol extract of *H. tuberculatum* was determined as described by El-Meligy *et al.*[17].

Antiulcerogenic activity: The evaluation of the anti-ulcerogenic activity was carried out using an absolute ethanol-induced ulcer model as described by Bighetti *et al.*[18]. Eighty-six male Wistar rats were divided into 5 groups, each of 6 rats, the rats of groups 1 and 2 received the vehicle (5 mL kg^{-1}) and served as normal control and ulcer control groups. Group 3 administered ranitidine at a dose of (100 mg kg^{-1}) and served as reference drug group. The induction of peptic ulcer was carried out using an absolute ethanol-induced ulcer method described by El-Meligy *et al.*[19] animals received an oral dose of absolute ethanol (1mL 200 g). One hour after inducing ulcer, groups 4, 5 received orally the first doses of the total alcohol extract (500 and 1000 mg kg^{-1}) separately. The rest of the doses were given to the animals orally once daily for 3 consecutive days. At the end of the experiment, animals were sacrificed, by inhalation, the stomachs were rapidly removed, opened along their greater curvature and gently rinsed under running tap water.

Lesion scores were quantified by the scoring system (0-5)[19]. Ulcer indices (mm) were calculated as the sum of the total length of long ulcers and petechial lesions in each group of rats divided by its number. The percentage of protection was determined according to the formula:

$$\text{Protection of control ulcer (\%)} = \frac{\text{Control UI-Test UI}}{\text{Control UI}} \times 100 \quad (1)$$

Anti-ulcerative colitis activity: Eighty-six male Wistar rats were divided into 5 groups, each of 6 rats, the rats of groups 1 and 2 received the vehicle (5 mL kg^{-1}) and served as normal control and ulcer control groups. Group 3 administered dexamethasone (0.1 mg kg^{-1}) and served as Reference Drug group. The Ulcerative colitis were induced by a slow infusion of 2 mL (4%, v/v) acetic acid in saline into the colon through the catheter[19]. Two hours after inducing colitis, groups 4, 5 received orally the first doses of the total alcohol extract (500 and 1000 mg kg^{-1}) separately. The rest of the doses were given to the animal orally once daily for 5 consecutive days. At the end of the excrement, animals were sacrificed, colonic segments (8 cm in length and 3 cm proximal to the anus) were excised, opened and were used for macroscopic scoring[20].

Assessment of colonic lesions: The colon specimens were weighted and wet weight/length ratio was calculated for all the rats. The specimens were examined under a dissecting microscope and the mucosal lesions were quantified by the scoring system (0-5) given Awaad *et al.*[21] after some modifications.

Sub-chronic toxicity: For carrying the sub-chronic toxicity, Male Wister rats were divided into groups each of 6 rats. The 1st group was left as a control and administrated with the vehicle orally, while the 2nd group orally administrated the total alcohol extract in a dose of 200 and 400 mg kg^{-1} for 15 days. After the examination period, 6 hours after the last dose blood samples were collected from the orbital plexus of rats. Samples were left to clot at room temperature for 30minutes then centrifuged at 1000 rpm for 20 min. The collected sera were used for the determination of the activity of both the aspirate aminotransferase (AST) and alanine aminotransferase (ALT)as liver markers. In addition, levels of blood urea, serum creatinine were also estimated as kidney markers[20], in addition to hematological parameters, Sodium, Potassium and Magnesium, Myloperoxidase activity (MPO).

The same process was used for the investigation of the same parameters after treatment for peptic ulcer and ulcerative colitis

Statistical analysis: All values were expressed as Mean±SD. Statistical analysis was done by using SPSS 10(IBM Corp. Released 2010 (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp). The Statistical significant differences between the two means were assessed by unpaired Student's' test. Differences at $p<0.05$, 0.01 and 0.001 were considered statistically significant[14].

RESULTS AND DISCUSSION

The phytochemical screening of *H. tuberculatum* showed that it contains/flavonoids, tannins, unsaturated sterols and/or triterpenoids, carbohydrates or glycosides and proteins and/or amino acids (Table 1) these groups are very common in

Rutaceae plants[1,6,9]. The presence of flavonoids, tannins, groups in this plant indicated the possible potential antioxidant[14] activity which played very important role in treatment of many diseases[22,23] specially gastrointestinal disorder[17,19].

Pharmacological activities

Acute toxicity (LD_{50}) test: The tested extract was characterized by a low degree of toxicity. The obtained results indicated that different doses of the alcohol extract *H. tuberculatum* (200 and 400 mg kg^{-1}) did not produce any symptoms of acute toxicity and none of the mice died during 24 h of observation. It was suggested that oral LD_{50} of the tested extracts were higher than 4000 mg kg^{-1}. Since substances possessing LD_{50} higher than 50 mg kg^{-1} are non-toxic[23], thus the tested extract is considered safe.

Anti-ulcerogenic activity: Ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity. Ulcers caused by ethanol are due to superficial damage to mucosal cells. The ethanol-induced ulcers are predominant in the glandular part of the stomach. It was reported that the ethanol stimulates the formation of mast cell secretary products[24].

Table 1: Preliminary phytochemical screening of *H. tuberculatum* (Forssk.)

Phytochemical	Present/absent
Alkaloids and/or nitrogenous bases	-
Anthraquinones	-
Carbohydrates or glycosides	+
Cardinolides	-
Flavonoids	+
Oxidase enzyme	-
Proteins and/or amino acids	+
Saponins	-
Tannins	+
Unsaturated sterols and/or triterpenoids	+
Volatile oil	-

(-): absent, (+): present

Gastric damage induced by absolute ethanol in the current study was characterized by both long ulcers and petechial lesions. The number of ulcers and the ulcer index in the control rats that received ethanol significantly increased when compared to the normal untreated animals. The total alcohol extract of *H. tuberculatum* (200 and 400 mg kg^{-1}) and Ranitidine (20 mg kg^{-1}) produced a significant ($p<0.05$) reduction in the ulcer index. Protection of control ulcer ranged from 28.30-38.00% as shown in Table 2.

Effect on ulcerative colitis: The model of acetic acid induced colitis shares many of the histologic features of ulcerative colitis in human beings including mucosal edema and sub-mucosal ulceration[19].

In the rats of Sham group, no abnormal changes were observed, suggesting that the handling procedure had no interference with the experimental outputs. Macroscopic damage parameters of the colon of control colitis rats, two days after rectal infusion of acetic acid revealed dark brown lesions, mucosal hyperemia, edema, erosion and ulceration. Control colitis rats show ed lesion score, ulcer area and ulcer index values of 4.8±0.77, 4.9±0.54 cm^2 and 9.9±0.82, respectively (Table 3).

The inflammatory changes of the intestinal tract were associated with a significant increase ($p\leq0.05$) of wet weight/length of the colon specimens as an indicator of inflammation. These inflammatory indices were significantly ($p<0.05$) improved by an oral dosing of dexamethasone, alcohol extract of *H. tuberculatum* and the isolated compounds for 5 days prior to ulcer induction. The effect of the extracts was dose dependent, the higher the dose the higher the cure of the diseases (42.74 and 31.51% for a dose of 400 and 200 mg kg^{-1}, respectively (Table 3).

Sub-chronic toxicity: Urea and creatinine are the most sensitive biochemical markers employed in the diagnosis of

Table 2: Effect of *H. tuberculatu* extract on absolute ethanol-induced ulcer rats

Extract	Dose (mg kg^{-1})	Score	No. of ulcers	Ulcer index	Curative (%)
Ulcer control	-	4.00	14.00±1.05	13.00±1.00	-
Ranitidine	100	2.20	7.60±3.05**	8.00±2.24*	33.30
Total extract	400	1.60	3.40*±2.51**	7.40±1.52*	38.00
Total extract	200	2.20	6.80±1.92**	8.60±2.51	28.30

Data are expressed as Mean±SD, n = 5, *$p\leq0.05$, **$p\leq0.01$

Table 3: Effects of *H. tuberculatu* alcohol extract on the macroscopic parameters of ulcerative colitis induced by acetic acid in rats

Groups	Lesion scores (0-5)	Ulcer area (cm^{-2})	Ulcer index	Wet W/L (g cm^{-1})	Curative (%)
Sham	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Control colitis	4.80±0.77	4.90±0.54	9.90±0.82	0.98±0.17	-
Dexamethasone (0.1 mg kg^{-1})	2.00±0.12***	2.40±0.15***	4.40±0.28***	0.39±0.02***	58.8
H. tuberculatum (400 mg kg^{-1})	2.98±0.52*	2.87±1.51*	4.70±1.90*	0.41±0.07*	42.74
H. tuberculatum (200 mg kg^{-1})	3.30±0.24*	3.01±0.27*	5.20±0.39*	0.52±0.04*	31.51%

Data are expressed as Mean±SD, n = 5, *$p\leq0.05$, ***$p\leq0.01$

Table 4: Effect of *H. tuberculatu* extracts on kidney function in normal, control, induced Peptic Ulcer (PU) and Ulcerative Colitis (UC) rats

	Parameters (mg dL^{-1})			
	Urea	Uric acid	Creatinine	Bilirubin
PU (plasma urea)				
Normal	17.33±1.52	6.63±0.410	0.7±0.10	0.53±0.05*
Control	29.66±2.08*	15.46±0.66 *	0.93±0.25*	1.1±0.30*
H. tuberculatum (400 mg kg^{-1})	28.63±1.92	12.73±0.95	0.45±0.1*	0.76±0.3*
H. tuberculatum (200 mg kg^{-1})	24.33±1.52	11.43±0.85	0.56±0.2*	0.66±0.2*
Ranitidine	17.66±1.52*	8.53±1.09*	0.53±0.12*	0.56±0.15*
UC (uric acid)				
Normal	19.66±1.52 *	4.36±1.05	0.36±0.11	0.26±0.05*
Control	35.66±2.08*	11.3±166 *	1.43±0.2*	0.86±0.05*
H. tuberculatum (400 mg kg^{-1})	29.11±2.88*	4.21±0.55 *	0.56±0.3*	0.52±0.55*
H. tuberculatum (200 mg kg^{-})	32.33±2.08*	6.56±0.75*	0.86±0.2*	0.66±0.25*
Dexamethasone (0.1 mg kg^{-1})	21.33±2.08*	4.83±0.41*	0.46±0.11*	0.33±0.05*

Data are expressed as Mean±SD, n = 5, *p≤0.05

Table 5: Effect of *H. tuberculatum* Extract on liver enzymes in normal, control, induced Peptic Ulcer (PU) and Ulcerative Colitis (UC) rats

	SGOT (U L^{-1})	SGPT (U L^{-1})	ALP (U L^{-1})
PU			
Normal	31.66±4.16	60.66±4. 5	52.33±4.50
In. control	68.66±5.5*	101±6.55*	124.66±6.02*
H. tuberculatum (400 mg kg^{-1})	30.26±7.03*	75.38±7.31*	90.43±8.1*
H. tuberculatum (200 mg kg^{-1})	34.66±7.02*	83.33±9.71*	82.33±9.6*
Ranitidine	27.66±4.04*	63.33±7.09*	67.66±4.0*
UC			
Normal	22.33±3.5	47.33±4.04	63.66±4.50
In. control	53.66±6.65*	90.66±6.11*	105.66±9.07*
H. tuberculatum (400 mg kg^{-1})	27±3.510*	61.22±11.51*	89.54±8.01*
H. tuberculatum (200 mg kg^{-1})	27±3.510*	56.66±10.01*	79.66±7.02*
Dexamethasone (0.1 mg kg^{-1})	22.66±3.05*	54.33±5.68*	64.33±7.5*

Data are expressed as Mean±SD, n = 5, *p≤0.05

renal damage. In kidney damage, retention of urea and creatinine in the blood is present[24], therefore, an increase in serum urea and creatinine as indications of functional damage to the kidney[25,26]. By these indicators, the control showed a significant increase (p<0.05) in plasma urea, uric acid, creatinine and bilirubin as compared to normal in PU. *H. tuberculatum* (200 mg kg^{-1}) extracts which significantly decreased (p<0.05) the level of plasma urea, uric acid, creatinine and bilirubin compared to the control. Standard drug Ranitidine also significantly reduced (p<0.05) the level of plasma urea, uric acid, creatinine and bilirubin to the normal level.

Control showed a significant increase (p<0.05) in plasma urea, uric acid, creatinine and bilirubin as compared to normal in UC. *H. tuberculatum* (200 mg kg^{-1}) extract which significantly decreased (p<0.05) the level of plasma urea, uric acid, creatinine and bilirubin compared to the control. Standard drug Dexamethasone also significantly reduced (p<0.05) the level of plasma urea, uric acid, creatinine and bilirubin to the normal level (Table 4).

Liver function parameter in control showed a significant increase (p<0.05) in the plasma SGOT, SGPT and ALP as compared to normal in PU. *H. tuberculatum* (200 mg kg^{-1}) extracts which significantly decreased (p<0.05) the level of plasma SGOT, SGPT and ALP compared to the control. Standard drug Ranitidine also significantly reduced (p<0.05) the level SGOT, SGPT and ALP to the normal level.

The control showed a significant increase (p<0.05) in plasma SGOT, SGPT and ALP as compared to normal in UC. *H. tuberculatum* (200 mg kg^{-1}) extract which significantly decreased (p<0.05) the level of plasma SGOT, SGPT and ALP compared to control. Standard drug Dexamethasone also significantly reduced (p<0.05) the level SGOT, SGPT and ALP to the normal level (Table 5). This finding indicate that this plant can be used safely to patient because the liver enzymes shown are approximately the same as the normal ones[27].

Control showed a significant decrease (p<0.05) in RBC, WBC, Hb and platelets as compared to normal in pu. *H. tuberculatum* (200 mg kg^{-1}) extract which significantly increased (p<0.05) RBC, WBC, Hb and platelets compared to the control. Standard drug Ranitidine also significantly increased (p<0.05) RBC, WBC, Hb and platelets to the normal level.

The control showed a significant decrease (p<0.05) in RBC, WBC, Hb and platelets as compared to normal in uc. *H. tuberculatum* (200 mg kg^{-1}) extract which significantly

Table 6: Effect of *H. tuberculatu* extract on hematological parameters in normal, control, induced peptic ulcer and ulcerative colitis rats

	RBC (10^{-6} mm^{-3})	WBC (10^{-3} mm^{-3})	Hb (g dL^{-1})	Platelets (10^3 mL^{-1})
PU				
Normal	9.15±0.38	9533.33±152.75	11.53±1.19	326.66±20.81
In. control	6.13±0.22*	5133.33±208.16*	6.8±0.70*	213.33±20.81*
H. tuberculatum (400 mg kg^{-1})	7.88±1.05*	6906.56±255.19*	8.53±0.8*	299.31±22.04*
H. tuberculatum (200 mg kg^{-1})	6.84±1.15*	5966.66±378.59*	7.23±0.9*	243.33±32.14*
Ranitidine	8.83±0.76*	8933.33±251.66*	9.83±0.45*	316.66±25.16[a]*
UC				
Normal	9.36±0.49	9633.33±152.75	11.26±0.85	323.33±15.27*
In. control	5.48±0.35*	4766.66±351.18*	7.76±0.51*	213.33±15.27*
H. tuberculatum (400 mg kg^{-1})	8.37±0.54*	7354.33±457.18*	9.13±0.23*	306.16±20.51*
H. tuberculatum (200 mg kg^{-1})	6.47±0.54*	6233.33±351.18*	9.53±0.35*	266.66±20.81*
Dexamethasone (0.1 mg kg^{-1})	8.02±0.14*	8033.33±602.77*	9.73±0.41*	316.66±25.16*

Data are expressed as Mean±SD, n = 5, *p = 0.05

Table 7: Effect of *H. tuberculatum* extract on sodium, potassium, and magnesium in normal, control, induced peptic ulcer and ulcerative colitis rats

	Units (mmol L^{-1})		
	Magnesium	Potassium	Sodium
PU			
Normal	132.66±9.070	6.86±0.70	2.8±0.360
In. control	136.33±9.07*	5.53±0.96*	1.3±0.450*
H. tuberculatum (400 mg kg^{-1})	131.46±22.34*	5.67±0.01*	2.06±0.27*
H. tuberculatum (200 mg kg^{-1})	134.66±12.34*	5.43±0.05*	1.66±0.25*
Dexamethasone (0.1 mg kg^{-1})	136.66±10.06*	6.06±0.58*	2.73±0.41*
UC			
Normal	151.33±3.05	6.33±0.45	2.36±0.15
In. control	123.33±4.50*	3.56±0.20*	0.93±0.15*
H. tuberculatum (400 mg kg^{-1})	120.13±3.06*	3.01±0.19*	1.00±0.30*
H. tuberculatum (200 mg kg^{-1})	129.33±3.05*	3.43±0.15*	1.30±0.20*
Dexamethasone (0.1 mg kg^{-1})	146.66±4.50*	5.36±0.35*	2.36±0.15

Data are expressed as Mean±SD, n = 5, *p = 0.05

Table 8: Effect *H. tuberculatum* extract on myeloperoxidase activity (MPO) of control and ulcerative colitis induced rats

	Myeloperoxidase (U mg^{-1})
Normal	4.83±0.32
In. control	22.33±1.52*
H. tuberculatum (400 mg kg^{-1})	17.63±0.30*
H. tuberculatum (200 mg kg^{-1})	14.13±0.20*
Dexamethasone (0.1 mg kg^{-1})	5.733±0.4*

increased (p<0.05) RBC, WBC, Hb and platelets compared to control. Standard drug Dexamethasone also significantly increased (p<0.05) RBC, WBC, Hb and platelets to the normal level (Table 6).

Comparing with control the plant extract a significant increase (p<0.05) in plasma sodium and a significant decrease (p<0.05) was noticed in plasma potassium and magnesium as compared to normal. The results showed that the plant extracts decrease the serum level of elements after treatment of rates from both PU & UC (Table 7).

Myeloperoxidase activity of *H. tuberculatum* extracts (400 and 200 mg kg^{-1}) was compared with in the control, it showed significant decrease (p<0.05) of myeloperoxidase. Also, Standard drug Dexamethasone was reduced (p<0.05), myeloperoxidase activity to the normal level (Table 8). The plasma MPO concentrations may be a marker of neutrophil proliferation and severity of inflammation[26]. The decrease of this enzyme in all treated rates is an indication that this plant has some activity in decreasing inflammations[28,29].

Mucin activity of gastric juice was affected by the plant extracts in both concentrations (400 and 200 mg kg^{-1}) in PU rats. Compared to normal. *H. tuberculatum* (400 and 200 mg kg^{-1}) extracts significantly decreased protein, hexose, hexosamine, sialic acid and fucose compared to the control. Standard drug Ranitidine also significantly reduced (p<0.05) all parameters less than the normal level (Table 9). The decreases which have been noticed in all parameters proved that the plant extract showed cyto-protective effects which is attributed to its flavonoidal contents[30].

From previous findings, it was clear that this *H. tuberculatum* can be used for the treatments of Peptic ulcer and ulcerative colitis with some limitation with people suffering from kidney diseases[28].

Table 9: Effect of *H. tuberculatum* extract on mucin activity of gastric juice in aspirin induced ulcers

	Units (μg mL^{-1})				
	Protein	Hexose	Hexosamine	Sialic acid	Fucose
Normal	263.13±11.48	365.00±8.52	214.83±7.64	66.66±2.59	56.83±1.30
In. control	358.90±4.09*	266.96±13.12*	121.90±2.4*	24.80±2.82*	18.03±1.8*
H. tuberculatum (400 mg kg^{-1})	341.71±11.64*	285.43±7.42*	154.16±6.76*	20.43±2.25*	31.23±2.04*
H. tuberculatum (200 mg kg^{-1})	338.21±11.64*	279.83±7.42 *	144.36±6.76*	29.93±2.25*	20.63±2.04*
Ranitidine	280.86±7.90*	354.63±6.865*	194.26±12.02*	55.76±3.16*	48.60±8.59*

CONCLUSION

Plant extract of *Haplophyllum tuberculatum* showed both potential antiulcer and anti-ulcerative colitis activities. The plant showed curative effect on peptic ulcer with dose dependent (28 and 38% for both 200 and 400 mg kg^{-1}, respectively). In case of treatments of ulcerative colitis the curative effect was dose dependent (31.51 and 42.74% for both 200 and 400 mg kg^{-1}, respectively). The plant also can be use as dietary supplements for minerals (Sodium, Potassium and Magnesium) deficiency because the results showed that the plant extracts decrease the serum level of elements after treatment of rates from both PU & UC. Mucin activity of gastric juice, protein, hexose, hexosamine, sialic acid and fucose were affected by the plant extracts (400 and 200 mg kg^{-1}) in PU rats and showed decrease in concentration comparing with control once which can prove that the plant extract showed cyto-protective effects which may be attributed to its flavonoidal contents. Myeloperoxidase activity was measured and showed some decrease (14.13±0.2 and 17.63±0.3 U mg^{-1} for both 200 and 400 mg kg^{-1}, respectively) lesser than controlled animals (22.33±1.52 U mg^{-1}) which indicates that the plant has anti-inflammatory activists.

SIGNIFICANCE STATEMENTS

This study discovers that *Haplophyllum tuberculatum* can be beneficial for the treatment of peptic ulcer and ulcerative colitis with some precautions for people with kidney problems. This study will help the researcher to uncover the critical areas of using a natural product in ruminations that many researchers were not able to explore. This plant has been used for many activities in folklore medicine without any previous scientific evidence and this work discovered a new activity for it.

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