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Research Article

EVALUATION OF EFFECT OF ETHANOLIC EXTRACT OF *FAGONIA CRETICA* L. PLANT AGAINST EXPERIMENTALLY INDUCED ULCERATIVE COLITIS IN MICE

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ABSTRACT

Fagonia cretica L. is conventional herb medication that is broadly utilized in clinical health care. The clinical functions of *Fagonia cretica* L. include immunomodulatory, anti-inflammation, anti-microbial, hepatoprotective activity. In spite of the fact its effects on inflammation of the colon remains undefined. We examined the effects of Ethanolic extract of *Fagonia creica* L. (EEFC) plant in dextran sulfate sodium (DSS) induced ulcerative colitis in mice. DSS (2%) was given orally in drinking water for 7 days to induce ulcerative colitis. Change in body weight, food intake, water intake was calculated every day. The degree and seriousness of ulceration was examined macroscopically using a disease activity index (DAI). After 9th day, colon mucosal damage index (CMDI), colon weight & length, myeloperoxidase (MPO), an exact marker of inflammation and antioxidants parameters (MDA, SOD, CAT) was evaluated. EEFC (400 mg/kg) significantly improved the body weight, colon length of the animals as compared to the disease control group. Animal treated with EEFC (400 mg/kg) showed significantly improved CMDI (1.40±0.27) & DAI (1.28±0.23) as compared to the disease control group CMDI (3.40±0.39), DAI (3.87±0.11). Higher level of SOD and Catalase and lower level of MDA were observed in animals treated with EEFC (400 mg/kg) as compared to disease control group. Animals treated with EEFC (400 mg/kg) significantly decreased in MPO level as compared to disease control group. The anti ulcerative effect of extract of *Fagonia cretica* L was comparable to sulphasalazine (100 mg/kg, p.o.) as standard. The outcomes recommended the defensive capability of EEFC in DSS-instigated colitis and this may be credited to its phytoconstituents that have been found to initiate a wide range of this study, for example, EEFC has substantial potential for the treatment of inflammatory colitis.

Keywords: Ulcerative colitis, DSS, Sulphasalazine, Ethanolic extract of *Fagonia cretica* L. (EEFC), Tissue-inflammation.

1. INTRODUCTION

Ulcerative colitis is inflammatory disease of the rectum and colon, which may ulcerate and become infected due to variable interaction with environmental related factors genetic susceptible factors. It is introduced by variety of clinical appearances, including bloody diarrhea, rectal bleeding, fever, weight loss, abdominal cramps, which may begin slowly or start totally all at once[1].

The conveyance of IBD shifts in geographical areas through high occurrence rate in North American and Northern Europe; at the same time, the morbidity stays stable in these regions. In the world, developing region like: Asia, the rate and morbidity are expanded every year [2].

Currently available therapeutic treatments *i.e.* 5-aminosalicyclic salisylates and its subsidiaries are as yet the medications of disease, immunosuppressant, and biological agents are utilized in increasingly serious types of the disease. But the therapies not only show limited advantages but also have genuine side effects [3].

It is common to use substitute medicine in ulcerative colitis, existence of various phytochemicals with potential benefits makes use of herbal drug a key choice in disease having poor prognosis with Allopathic medicines. For a considerable length of time, herbal remedies have been utilized for human disease executives because plants contain large number of new components of various therapeutic actions. *Fagonia cretica* L. (Zygophyllaceae) is a traditional herb praised with rich phytochemicals like: flavonoids: (kaempferol, quercetin, isorhamnetin 3-rutinoside, herbacetin 8-rutinoside), triterpenoids: (oleanolic acid & ursolic acid), saponins etc. [4]. They are customarily notable for the treatment of inflammation, open wounds, hemorrhoids, leprosy, sores and fever in the form of different conventional formulation [5]. Kaempferol is reported to possess,

suppress inflammation in dextran sulphate sodium (DSS) induce ulcerative colitis in mice [6]. Quercetin is reported to inhibit inducible nitric oxide synthase (iNOS) expression in DSS & TNBS induce ulcerative colitis and is reported to inhibit NF-kB transcription factor in murine intestinal epithelial cells [7]. Oleanolic acid present in *Fagonia cretica* inhibit NF-kB signalling pathway in DSS induced ulcerative colitis in mice [8]. Ursolic acid protects against ulcerative colitis via anti-inflammatory and antioxidant effect in mice [9].

Additionally, current researches reveal that *Fagonia cretica* L. has several biological functions such as anti-inflammatory, analgesic, antitumor, antipyretic [10], immunomodulatory [11], antioxidant [12], anti-microbial [13], antibacterial [14] and Hepatopretective activity [15]. In the current study, the effect of ethanolic extract of *Fagonia cretica* L. plant against experimentally induced ulcerative colitis in mice was evaluated.

2. MATERIAL AND METHODS

2.1. Plant collection and Authentication

Whole plant was collected from Sasoi dam near Jamnagar, Gujarat and authentication of plant was done at Department of Botanical Science, School of Science R.K. University, Rajkot.

2.2. Plant extraction

Whole Plant was shade dried; approximately (500 g) was powdered to coarse particle size and extracted with 80% ethanol by maceration method for 5 days. Solvent was evaporated using rotary evaporator apparatus and concentrated to dryness at 40°C [16].

2.3. Phytochemical Screening

Phytochemical anlysis was done in the *Fagonia cretica* L. for checking the occurrence of flavonoids, triterpenoids, steroids, alkaloids, cardiac glycosides, carbohydrates, tannins, saponins, amino acid and protein [17].

2.4. TLC (Thin layer chromatography)

TLC was performed for identification of four major constituent's kaempferol, quercetin, oleanolic acid, ursolic acid in ethanolic extract of *Fagonia cretica* [18, 19].

2.5. LC-MS analysis

Ethanolic extract of *Fagonia cretica* L. was analyzed by using LC-MS for presence of (oleanolic acid, ursolic acid & kaempferol) content, Chromatographic conditions [20, 21].

Table 1: Specifications for LC/MS

Parameters	Specifications
Column	C18 column (4.6 × 100 mm, 3 µm)
Mobile Phase	Mobile phase A: 0.1%formic acid in water Mobile phase B: 0.1% formic acid in acetonitrile
Flow rate	1.0 mL/min
Column oven temperature	30°C
Auto sampler temperature	350°C
Volume of injection	10 µl
Detector	ESI-MS
Detection wavelength	280
Run time	90 min

2.6. Experimental animals

BALB/c mice (25-30g) of either sex were used. They were housed at temperature (22±1°C), relative humidity (30-70%) and 12h/12h light dark cycle and provide pellet diet and purified drinking water. Experiment was conducted according to the CPCSEA guideline and the study was approved by the Institutional Animal Ethics Committee (Protocol no: BKMGPC/IAEC24/RP63/2019).

2.7. Induction of ulcerative colitis

Dextran sulfate sodium (DSS) was utilized for the enlistment of ulcerative colitis as depicted already [22]. The drinking water for mice was supplanted by 2% DSS arrangement and mice got DSS orally for 7 days. On 8th day, animals were killed and colonic parts were taken for macroscopic and other parameters examinations.

2.8. Study design

Animals were divided in six groups, each group had 6 animals. *Group I (normal control):* mice received only vehicle (0.5% CMC, p.o.) daily for 9 days. On 10th day mice were killed and macroscopic and other parameters were performed. *Group II (Disease control):* 2% DSS was given orally to the mice for 3rd-9th days to produce colitis. On 10th day mice were killed and macroscopic and other parameters were performed. *Group III (Standard control):* 0.5% CMC daily for 9 days with sulfasalazine (100mg/kg) daily for 9 days. On 10th day mice were killed and macroscopic and other parameters were performed. *Group IV (EEFC 100 mg/kg):* 100 mg/kg EEFC was administered daily for 9 days + 2% DSS for 3rd-9th days. On 10th day mice were killed and

macroscopic and other parameters were performed. *Group V (EEFC 200 mg/kg):* 200 mg/kg EEFC was administered daily for 9 days + 2% DSS for 3rd-9th days. On 10th day mice were killed and macroscopic and other parameters were performed. *Group VI (EEFC 400 mg/kg):* 400 mg/kg EEFC was administered daily for 9 days + 2% DSS for 3rd-9th days. On 10th day mice were killed and macroscopic and other parameters were performed [23].

[EEFC: Ethanolic extract of Fagonia cretica L. CMC: carboxymethylcellulose, p.o. = per oral]

2.9. Evaluation parameters

2.9.1. Measurement of body weight

Weights of animals were observed every day from 1-9 days the % change in body weight was considered by dissimilarity between initial 7 final body weights of animal [24].

2.9.2. Measurement of Food & Water intake

The food and water intake was measured every day and % difference was calculated on 10th day [25-26].

2.9.3. Measurement of Disease activity index

Score is specified for change in body weight, rectal bleeding and stool consistency from which disease activity index was calculated [27].

Formula for DAI = change in weight + stool consistency (diarrhea) + score of rectal bleeding

Table 2: Calculation of disease activity index score (DAI)

Score	Wight loss	Stool constituency	Bleeding
0	None	Normal	Normal
1	1- 4.99%	Slightly loose feces	Slightly bloody
2	5-10%	Loose faces	Bloody
3	> 10%	Watery diarrhea	Blood in whole colon

2.9.4. Measurement of colon length & weight

At the end of day 9 day mice were sacrificed. The colon was removed by dissection and length of colon was calculated by keeping the colon on a graph paper and by checking the length using centimeter as standard. Sensitive weighing balance was used for measurement of colon weight [28].

2.9.5. Measurement of Colon mucosal damage index (CMDI)

Colon segment approximately 10 cm was excised, rinsed using saline and open into midline. Macroscopic scoring was done [29].

Table 3: Score for CMDI (colon mucosal damage index)

Score	Damage
0	Normal mucosa
1	Mild hyperaemia and oedema, on mucosal part of colon no erosion or ulcer
2	Moderate hyperaemia and oedema, on mucosal part of colon erosion observed
3	Severity of hyperemia, edema and necrosis noted and on mucosal surface ulcer is observed with ulcerative area <1 cm
4	Severity of hyperaemia and edema, necrosis noted & on mucosal surface ulcer is noted with ulcerative area >1 cm

2.9.6. Determination of myeloperoxidase (MPO) activity

Polymorphonuclear cell infiltrate so inflammatory injury produced into the colon. This activity was checked by MPO. The colon was removed and washed with saline solution and then homogenized with PPB (potassium phosphate buffer) consisting of DTAB. Homogenate was frozen and centrifuged at 4000 rpm for 20 min to verify the MPO activity. O-dianisidine (50mg) dye, 3 ml methanol + dye, Dye solution 1 ml + PPB solution 100 ml + 16.7 µl substrate+3% H_2O_2 were mixed. In cuvette 0.1 ml of homogenate was added and absorbance was checked using double beam spectrophotometer. Absorbance changes were recorded at 1 min for 15 sec intervals. This absorbance was taken at 460 nm at room temperature. 1 unit of MPO activity was defined as that degrading 1µmole of peroxidases per minute at 25°C. MOP activity was measured using the following formula [30]:

MPO (U/mg) = Absorbance per min ×10X

Where, X= weight of tissue taken for homogenate ×volume of supernatant taken for final reaction

2.10. Antioxidant studies

2.10.1. Determination of Malondialdehyde (MDA) activity

The level of Malondialdehyde (MDA) in the colon was determined as an indicator of lipid peroxidation. Cell membrane lipids peroxidation end product is malon-

dialdehyde and is measured as a dependable symbol of inflammatory tissue injury and reaction of malon-dialdehyde+2-thiobarbituric acid, chromogen will be obtained. 0.5 ml colon homogenate + 0.5 ml (20%) TCA + 1 ml of (0.67%) TBA were mixed. Boiled the mix at 100°C for 15 min then cooled straight away in ice and n-butanol (4 ml) was added. Centrifugation for 15 min at 3000 rpm was done and supernant absorbance was calculated at 530 nm using spectrophotometer and was compared with a curve made by MDA standard. Results were expressed as U/mg protein [31].

2.10.2. Determination of Super oxide dismutase (SOD) activity

Capacity to decrease the autoxidation of epinephrine by SOD at alkaline pH was measured. Tissue homogenate (0.2 ml) + (0.05 M) 2.5 mL carbonate buffer, (0.3 mM) 0.3 ml newly prepared adrenaline were added for starting the reaction. Absorbance was noted at 480 nm every 30s for 150s. One unit of SOD movement instigated around half restraint of the auto-oxidation of adrenaline to adenochrome in 1 min. Result was expressed as U/mg protein [32].

2.10.3. Determination of Catalase (CAT) activity

H_2O_2 shows stable boost in absorption with declining wavelength in the UV range. Catalase activity can be indicated by dissimilarity in the absorbance per unit time at 240nm. 50 µL of homogenate +2.95 mL of H_2O_2 were mixed and the absorbance was taken for 2 min in 1 min interval at 240 nm by UV-visible spectro-photometer.

Catalase activity of 1 unit was calculated using [33]:

$k = (2.303/\Delta t) \times \log (A1/A2)$,

Where k is rate constant of first order reaction

Δt: in min time interval, A1: At 1 min absorbance, A2: At 2 min absorbance.

2.10.4. Determination of total protein

Folin-Ciocalteu phenol reagent with NaOH was titrated to a phenolphthalein end-point. Based on this titration diluted the Folin reagent (around 2-crease) to make it 1 N in acid. These thus might be checked against a standard solution of crystalline bovine albumin. 0.2 ml of protein sample in test tube was added with 1 ml alkaline copper solution. Mixed properly and allowed to stand for 10 min at room temperature. 0.1 ml of diluted folin reagent was added very rapidly and mixed. After 30 min, sample was read in spectrophotometer [34].

2.11. Statistical analysis

Data was expressed as mean±SEM. Significant differences was detected using one-way analysis of variance (ANOVA) followed by post-test using computer based fitting program (Graph pad prism 8.4.2) for multiple comparisons. $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Plant extraction

The extraction of dry powder of whole plant of *Fagonia cretica* L. was carried out using ethanol for 5 days by maceration method. The extract was obtained in dark brown color and semisolid in nature. % yield of Ethanolic extract of 500gm dried powder of *Fagonia cretica* L. was found to be 7.3 %w/w using maceration method of extraction which is nearer to the reported in article [35].

3.2. Phytochemical screening

Preliminary phytochemical screening of Ethanolic extracts of *Fagonia cretica* L. was carried out and shown to contain phytoconstituents as reported in table 1.

Whole plant of *Fagonia cretica* L. has been reported to include a variety of phytochemicals. Preliminary phyto-chemical studies of ethanolic extract of whole plant of *Fagonia cretica* L. has been established to show presence of a choice of phytochemicals like flavonoids, tannins, steroids & terpenoids, saponins, cardiac glycoside, alkaloids, carbohydrate and proteins.

Table 4: Result of preliminary phytochemical tests of EEFC extract

Phytochemicals	Chemical test	Result
Flavonoids	Shinoda test	+
	Alkaline reagent test	+
Tannins	Ferric chloride test	+
Steroids & terpenoids	Salkowski test	+
	Liebermann-Burchard test	+
Saponins	Foam test	+
Cardiac glycoside	Killer killiani test	+
Alkaloids	Dragendroff's test	+
	Wagner's test	+
	Hager's test	+
Carbohydrate	Molisch's test	+
	Benedict's test	+
Proteins	Biuret test	+
	Million's test	+

These data support earlier reported phytochemical analysis of ethanolic extract of *Fagonia cretica* L. Present study was considered on the basis of presence of phytochemicals *i.e.* quercetin & kaempferol (flavonoids) and ursolic acid & oleanolic acid (Triterpinoids). Further confirmation of these components was carried out by performing TLC and LC-MS.

3.3. TLC analysis of Ethanolic extracts of *Fagonia cretica* L. plant

TLC analysis of plant extract showed presence of main chemical constituents like kaempferol, quercetin, ursolic acid & oleanolic acid. The R_f value of chemical constituents closer to the R_f values of standard which has been reported in earlier articles.

3.4. LC-MS analysis of Ethanolic extracts of *Fagonia cretica* L. plant

Ethanolic extract of *Fagonia cretica* L. was analyzed by using LC-MS for presence of ursolic acid, oleanolic acid & kaempferol. Mass data for chemical constituents present in Ethanolic extract of *Fagonia cretica* L. extract are as follows:

PDA chromatogram of ursolic acid (2.1), oleanolic acid (2.1) & kaempferol (3.5) present in Ethanolic extract of *Fagonia cretica* L. are shown in Fig. 1.

MS spectrum of ursolic acid & oleanolic acid (456.7 g/mol) present in Ethanolic extract of *Fagonia cretica* L. is shown in Fig. 2.

MS spectrum of kaempferol (286.23 g/mol) present in Ethanolic extract of *Fagonia cretica* L. is shown in Fig.3.

Table 5: Results of TLC analysis of EEFC extract

Compound	Kaempferol & Quercetin	Oleanolic acid & Ursolic acid
Mobile phase	Toluene-acetone-formic acid (7:3:0.25)	light petrol: ethyl acetate-acetone (8.2:1.8:0.1)
Spraying reagent	Natural product-polyethylene glycol reagent (NP/PEG)	H_2SO_4/diethyl ether reagent
Identification	Blue colour spot	Blue colour spot
R_f value (Std)	0.46& 0.60	0.31 & 0.15
R_f value (plant extract)	0.43 & 0.58	0.30 & 0.13

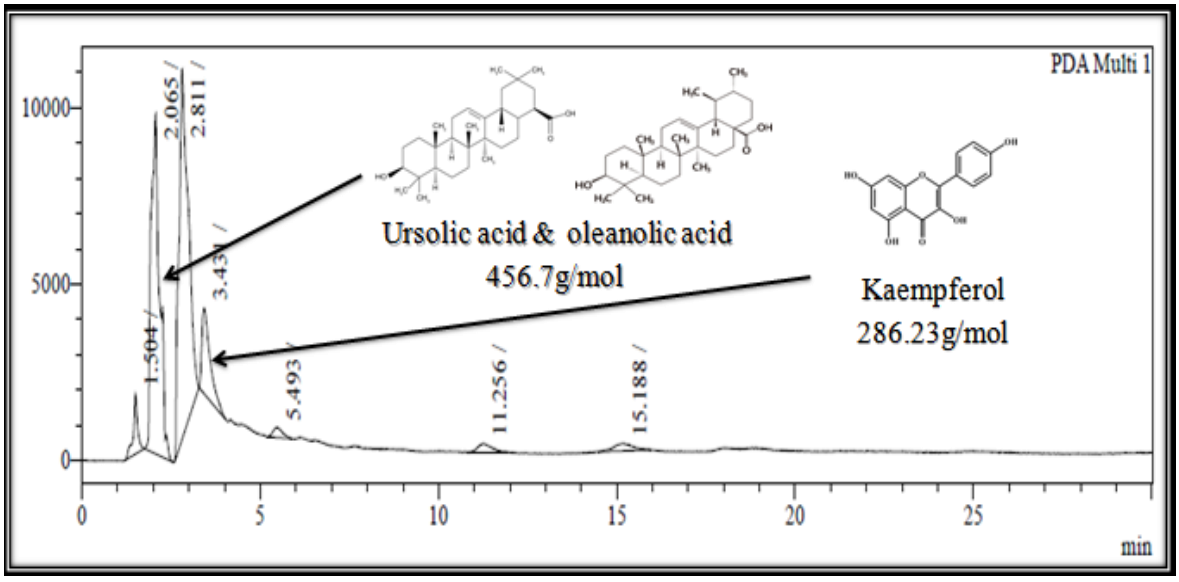

Fig. 1: Liquid chromatogram of Ethanolic extract of *Fagonia cretica* L. using C18 Column and 0.1% formic acid in acetonitrile as eluent

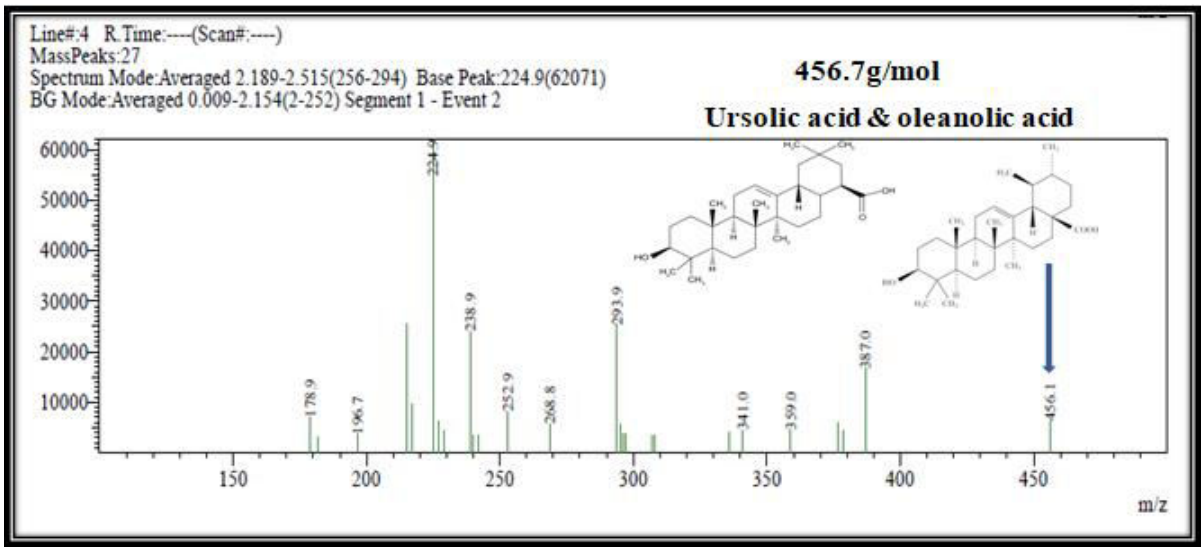

Fig. 2: Mass peak of ursolic acid & oleanolic acid (456.7g/mol) present in Ethanolic extract of *Fagonia cretica* L.

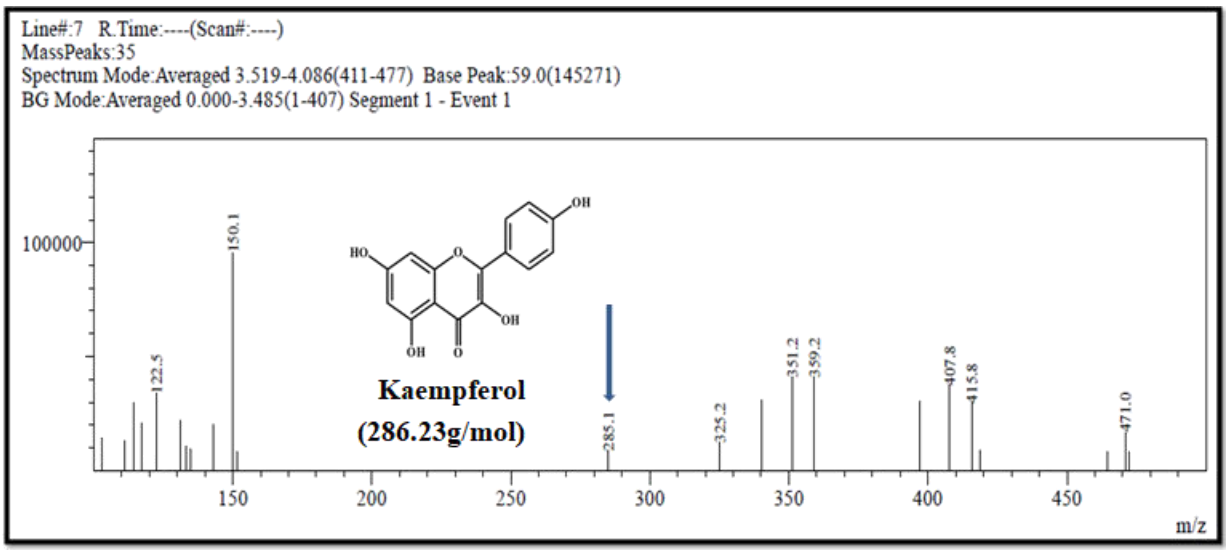

Fig. 3: Mass peak of kaempferol (286.23g/mol) present in Ethanolic extract of *Fagonia cretica* L.

LC-MS analysis showed the occurrence of ursolic acid and oleanolic acid at m/z- 456.1 and kaempferol at m/z- 285.1 which matches with their molecular weight. This data confirms the presence of ursolic acid, oleanolic acid and kaempferol in EEFC. Additionally, oleanolic acid is reported to inhibit NF-kB signalling pathway in DSS induced ulcerative colitis in mice and ursolic acid is reported to protect against ulcerative colitis via anti-inflammatory and antioxidant effect in mice and kaempferol is reported to possess, suppress inflammation in dextran sulphate sodium (DSS) induce ulcerative colitis in mice. Hence ethanolic extract of *Fagonia cretica might* produce anti-inflammatory effect in DSS induced ulcerative colitis due to presence of ursolic acid, oleanolic acid and kaempferol.

3.5. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on body weight

At the end of DSS administration (2%w/v), there was significant reduction in body weight of disease control group (21.68±0.26) as compared to normal control group (0.00±0.00, $p < 0.05$). Animal treated with standard drug sulphasalazine (100mg/kg) showed significant reduction in body weight (10.53±0.11) as compared to disease control group ($p < 0.05$). Animal treated with EEFC (200 mg/kg & 400 mg/kg) showed significant reduction in body weight (13.90±0.13 &

12.68±0.14) respectively as compared to disease control group ($p< 0.05$).

Table 6: Effect of Ethanolic Extract of *Fagonia cretica* L. Plant on body weight

Groups	Reduction in Body weight (gm)
Normal control	0.00±0.00
Disease control	21.68±0.26#
Std. treatment (sulphasalazine)	10.53±0.11*
EEFC treatment (100mg/kg)	19.87±0.12
EEFC treatment (200mg/kg)	13.90±0.13*
EEFC treatment (400mg/kg)	12.68±0.14*

*All the values are expressed in mean ± SEM (n= 6), # Significantly different from normal control ($p < 0.05$), *Significantly different from disease control ($p < 0.05$)*

The body weight was monitored every day in between 10.00 am to 11.00 am. Loss of body weight was calculated as difference of initial & final body weight of animal. In ulcerative colitis, immune cell infiltrate, they produce pro-inflammatory cytokines in response to activated Th1 & Th2 cells and induced tissue damage & hence it cause bleeding, diarrhea & loss of body weight. So weight loss may occur as a result of diarrhea and loss of appetite due to abdominal pain, nausea, vomiting or worsening diarrhea.

3.6. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on Food intake

At the end of 3-9 days of DSS administration (2%w/v). Food intake in disease control group (50.01±1.46) was was significantly decrease as compared to normal control group (0.00±0.00, $p<0.05$). Significant difference was also shown in standard drug sulphasalazine (28.00±1.00) as compared to disease control group (50.01±1.46, $p< 0.05$). Animal treated with EEFC (400 mg/kg) showed significant reduction in food intake (34.37±0.72) as compared to disease control group ($p< 0.05$).

In illness condition not completely processed food that moves through the colon may cause looseness of the bowels and stomach distress. In ulcerative colitis, the small digestive system works regularly, however the inflamed colon doesn't take in water appropriately, bringing about in diarrhea, increased urgency to have a bowel movement and increased frequency of bowel movements thus decrease food intake in DSS treated animals.

Table 7: Effect of Ethanolic extracts of *Fagonia cretica* L. Plant on Food intake

Groups	% inhibition in food intake (gm)
Normal control	0.00±0.00
Disease control	50.01±1.46#
Std. treatment (sulphasalazine)	28.00±1.00*
EEFC treatment (100mg/kg)	45.68±0.98
EEFC treatment (200mg/kg)	40.78±0.99
EEFC treatment (400mg/kg)	34.37±0.72*

3.7. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on water intake

At the end of 3-9 days of DSS administration (2%w/v), there was significantly lesser water intake in disease control group (55.00±2.01) as compared to normal control group (0.00±0.00, $p< 0.05$). Animal treated with standard drug sulphasalazine (100mg/kg) showed significantly higher water intake (25.00±3.00) as compared to disease control group ($p< 0.05$). Animal treated with EEFC (200 mg/kg & 400 mg/kg) showed significantly higher water intake (40.01±1.01 & 32.41±3.08) respectively as compared to disease control group ($p< 0.05$).

In normal body absorbance of nutrients, salts and water are necessary to provide energy. Due to injury of intestinal wall by DSS it's failed to absorb important salts and water thus water intake decrease in disease control animals.

Table 8: Effect of Ethanolic extracts of *Fagonia cretica* L. Plant on Water intake

Groups	% reduction in water intake (ml)
Normal control	0.00±0.00
Disease control	55.00±2.01#
Std. treatment (sulphasalazine)	25.00±3.00*
EEFC treatment (100mg/kg)	47.00±2.08
EEFC treatment (200mg/kg)	40.01±1.01*
EEFC treatment (400mg/kg)	32.41±3.08*

3.8. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on DAI (Disease Activity Index) Score

The body weight was monitored every day in between 10.00 am to 11.00 am from 3rd-9th days. Stool constituency & rectal bleeding were monitored daily in between 11.00am to 12 pm. At the end of 3rd-9th days of

DSS administration (2%w/v), there was significantly increase in DAI of disease control group ($p< 0.05$) as shown in table 9. Animal treated with standard drug sulphasalazine (100mg/kg) showed significantly decreased DAI as compared to disease control group ($p< 0.05$). Animal treated with EEFC (400 mg/kg) showed significantly decreased DAI as compared to disease control group ($p< 0.05$)

Table 9: Effect of Ethanolic Extract of *Fagonia cretica* L. plant on DAI Score

Days	GROUPS					
	Normal control	Disease control	Standard (100mg/kg)	EEFC (100mg/kg)	EEFC (200mg/kg)	EEFC (400mg/kg)
5	0±0.0	1.99±0.20	0.53±0.13	1.75±0.29	1.16±0.15	0.58±0.13
6	0±0.0	2.86±0.29	0.96±0.20	2.50±0.49	1.74±0.19	1.22±0.21
7	0±0.0	3.20±0.20	1.12±0.20	2.87±0.35	2.38±0.35	1.24±0.23
8	0±0.0	3.52±0.15	1.21±0.24	3.26±0.32	2.62±0.24	1.26±0.18
9	0±0.0	3.87±0.110#	1.36±0.17*	3.53±0.29	2.09±0.31*	1.28±0.23*

After 3rd day onwards, medium bleeding, change in body weight and change in stool was observed in DSS administered groups. Immune cells like macrophages and lymphocytes are infiltrating in the condition of ulcerative colitis. Th1 & Th2 are differentiated by the macrophage and they produced cytokines which produce damage into the tissues so cause diarrhea, severity in bleeding and decrease body weight. DAI was calculated on through the diarrhea score, bleeding score and decrease in body weight at 3rd to 9th day and was found significant changes in DAI from day 3 to 9.

3.9. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on Colon length

At the end of DSS administration (2%w/v), Colon length of disease control group (6.70±0.20) was significantly decreased as compared to normal control group (10.20±0.07, $p< 0.05$). Significant difference was also shown in standard drug sulphasalazine (9.29±0.08) as compared to disease control group (6.70±0.20, $p< 0.05$). Animal treated with EEFC (200mg/kg & 400 mg/kg) showed significantly higher colon length (7.56±0.11 & 8.64±0.05) respectively as compared to disease control group (6.70±0.20, $p< 0.05$).

Table 10: Effect of Ethanolic extracts of *Fagonia cretica* L. Plant on Colon length

Groups	Colon length (cm)
Normal control	10.20±0.07
Disease control	6.70±0.20#
Std. treatment (sulphasalazine)	9.29±0.08*
EEFC treatment (100mg/kg)	7.10±0.15
EEFC treatment (200mg/kg)	7.56±0.11*
EEFC treatment (400mg/kg)	8.64±0.05*

In the present study, mice having shortened colon length and increase the inflammation in disease control group, while EEFC (400 mg/kg) pre-treated group showed protective effect on colon length and inflammation.

3.10. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on Colon weight

At the end of 3-9 days of DSS administration (2%w/v), there was significant increase in colon weight of disease control group (0.36 ±0.0007) as compared to normal control group (0.29±0.008, $p< 0.05$). Animal treated with standard drug sulphasalazine (100mg/kg) showed significantly decrease colon weight (0.30±0.006) as compared to disease control group ($p< 0.05$). Animal treated with EEFC (200mg/kg & 400 mg/kg) showed significantly decreased colon weight (0.32±0.004 & 0.31±0.003) respectively as compared to disease control group ($p< 0.05$).

In present study mice having higher colon weight in disease control group due to presence of edema in inflamed colon, while EEFC (400 mg/kg) pre-treated group showed protective effect on colon weight & inflammation as shown in Fig. 11.

Table 11: Effect of Ethanolic extracts of *Fagonia cretica* L. Plant on Colon weight

Groups	Colon Weight (mg)
Normal control	0.29±0.008
Disease control	0.36±0.0007#
Std. treatment (sulphasalazine)	0.30±0.007*
EEFC treatment (100mg/kg)	0.34±0.006
EEFC treatment (200mg/kg)	0.32±0.004*
EEFC treatment (400mg/kg)	0.31±0.003*

3.11. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on Colon mucosal damage index score

At the end of 3-9 days of DSS administration (2%w/v), CMDI score in disease control group (3.40±0.39) was significantly higher as compared to normal control group (0.00±0.00, p< 0.05). Significant difference was also shown in standard drug sulphasalazine (1.10±0.19) as compared to disease control group (3.40±0.39, p< 0.05). Animal treated with EEFC (200 mg/kg & 400 mg/kg) showed significantly lower CMDI (2.31±0.29 & 1.40±0.27) as compared to disease control group (p< 0.05).

Table 12: Effect of Ethanolic extracts of *Fagonia cretica* L. Plant on Colon mucosal damage index score

Groups	CMDI Score
Normal control	0.00±0.00
Disease control	3.40±0.39#
Std. treatment (sulphasalazine)	1.10±0.19*
EEFC treatment (100mg/kg)	3.14±0.20
EEFC treatment (200mg/kg)	2.13±0.29*
EEFC treatment (400mg/kg)	1.40±0.27*

At the end of study periods, macroscopic analysis was done for colon tissue; oedema and inflammation in colon tissue and formation of lesion in mucosal layer was observed. In above table score of CMDI was showed higher in disease control groups of animals and ethanolic extract of *Fagonia cretica* L. 200 and 400 mg/kg dose gives better effect in CMDI score in current work.

3.12. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on Myeloperoxidase (MPO) activity

At the end of DSS administration (2%w/v), there was significant increase in MPO level of disease control group (2.90±0.15) as compared to normal control group (1.63±0.12, p< 0.05). Animal treated with standard drug sulphasalazine (100mg/kg) showed significant decrease in MPO level (1.66±0.13) as compared to disease control group (p< 0.05). Animal treated with EEFC (200mg/kg & 400mg/kg) showed significant decrease in MPO level (1.18±0.14 & 1.96±0.12) as compared to disease control group (p< 0.05).

Table 13: Effect of Ethanolic extracts of *Fagonia cretica* L. Plant on Myeloperoxidase (MPO) activity

Groups	MPO (U/ mg of protein)
Normal control	1.63±0.12
Disease control	2.90±0.15#
Std. treatment (sulphasalazine)	1.66±0.13*
EEFC treatment (100mg/kg)	2.50±0.11
EEFC treatment (200mg/kg)	1.18±0.14*
EEFC treatment (400mg/kg)	1.96±0.12*

Inflammation is occurs into the colon tissue due to polymorphnuclear (PMN) cells are infiltrate so inflammatory conditions are measured through activity of MPO. Chronic inflammation is occurs in to ulcerative colitis because of polymorphnuclear cells migrate into the colon tissue. In disease control groups MPO level shown high because of polymorphnuclear cells migrate to colon and cause injury of cell. Ethanolic extract of *Fagonia cretica* L. 400 mg/kg dose reduces MPO level. Reduced MPO level in Ethanolic extract of *Fagonia cretica* L. 400 mg/kg dose might reduce inflammatory mediators due to phytochemicals are present in this extract like kaempferol and quercetine.

3.13. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on Malondialdehyde (MDA) level

At the end of DSS administration (2%w/v), MDA level in disease control group (40.12±1.37) was significantly decreased as compared to normal control group (20.22±2.56, p< 0.05). Significant difference was also shown in standard drug sulphasalazine (23.02±2.54) as compared to disease control group (40.12±1.37, p< 0.05). Animal treated with EEFC (200 mg/ kg & 400 mg/kg) showed significantdecrease in MDA level (31.62±2.45 & 26.01±.1.35) as compared to disease control group (p< 0.05).

Table 14: Effect of Ethanolic extracts of *Fagonia cretica* L. Plant on MDA level

Groups	MDA level(U/ mg of protein)
Normal control	20.22±2.56
Disease control	40.12±1.37#
Std. treatment (sulphasalazine)	23.02±2.54*
EEFC treatment (100mg/kg)	36.78±1.76
EEFC treatment (200mg/kg)	31.62±2.45*
EEFC treatment (400mg/kg)	26.01±.1.35*

Reactive oxygen species & free radicals produce injury in mucosa layer so lipid peroxidation occurs. It is measured by substance like: thiobarbituric acid. During peroxidation of lipids malondialdehyde was produced which is indicated through thiobarbituric acid. Ethanolic extract of *Fagonia cretica* L. are reported to having antioxidant activity and also EEFC 400 mg/kg decrease MDA level in present work so this extract might give antioxidant activity due to constituents like quercetine and kaempferol.

3.14. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on Superoxide dismutase (SOD) level

At the end of 3-9 days of DSS administration (2%w/v), there was significant decrease in SOD level of disease control group (21.82±2.66) as compared to normal control group (66.75±5.76, $p< 0.05$). Animal treated with standard drug sulphasalazine (100mg/kg) showed significantly increase in SOD level (46.02±5.30) as compared to disease control group ($p< 0.05$). Animal treated with EEFC (200 mg/kg & 400 mg/kg) showed significant increase in SOD level (33.04±2.64 & 39.48±3.40) as compared to disease control group ($p< 0.05$).

Table 15: Effect of Ethanolic extracts of *Fagonia cretica* L. Plant on SOD level

Groups	SOD level (U/ mg of protein)
Normal control	66.75±5.76
Disease control	21.82±2.66#
Std. treatment (sulphasalazine)	46.02±5.30*
EEFC treatment (100mg/kg)	26.02±3.31
EEFC treatment (200mg/kg)	33.04±2.64*
EEFC treatment (400mg/kg)	39.48±3.40*

SOD converts the superoxide anion to H_2O_2 & O_2 then catalase convert H_2O_2 to H_2O and O_2. In disease condition, mucosa layer of intestine have antioxidant activity and first enzyme is SOD. Ethanolic extract of *Fagonia cretica* L. 400 mg/kg increased super oxide dismutase level in compared of disease control groups in current work. In intestinal endothelial cells leucocytes are bind but increased level of SOD prevents the adhesion of these leucocytes because SOD binds with this site of adhesion. SOD reduces the ROS level and stress so SOD gives anti-inflammatory activity in UC.

3.15. Effect of Ethanolic extracts of *Fagonia cretica* L. plant on Catalase (CAT) level

At the end of DSS administration (2%w/v), CAT level in disease control group (0.46±0.016) was significantly decreased as compared to normal control group (1.50±0.44, $p< 0.05$). Significant difference was also shown in standard drug sulphasalazine (1.43±0.03) as compared to disease control group (0.46±0.016, $p< 0.05$). Animal treated with EEFC (400 mg/kg) showed significant increase in CAT level (1.20±0.03) as compared to disease control group ($p< 0.05$).

In sub cellular organs like peroxisome, catalase enzyme is present and it contain heme group. Catalase enzyme converts H_2O_2 to H_2O & O_2. Both enzymes like catalase & super oxide dismutase have antioxidant activity and mechanisam. SOD converts the superoxide anion to H_2O_2 & O_2 while catalase converts this H_2O_2 to H_2O & O_2. Ethanolic extract of *Fagonia cretica* L. 400 mg/kg increase catalase level in compare of disease control groups due to presence of quercetine & kaempferol in this extract of current work.

Table 16: Effect of Ethanolic extracts of *Fagonia cretica* L. Plant on CAT level

Groups	CAT level (U/ mg of protein)
Normal control	1.50±0.04
Disease control	0.46±0.016#
Std. treatment (sulphasalazine)	1.43±0.03*
EEFC treatment (100mg/kg)	0.89±0.02
EEFC treatment (200mg/kg)	1.11±0.04
EEFC treatment (400mg/kg)	1.20±0.03*

4. CONCLUSION

In conclusion, the present investigation suggests that the *Fagonia cretica* L. plant extracts contain significant amounts of flavonoids and triterpenoids and exhibit strong anti-inflammatory, antioxidant, antilipid peroxidation and free radical scavenging activities. LC-MS data confirms the presence of kaempferol; ursolic acid and oleanolic acid in Ethanolic extract of *Fagonia cretica* L. plant which may give protective treatment in ulcerative colitis. The animal treated with EEFC (400 mg/kg) showed significantly higher colon length. While, CMDI and DAI were significantly lower as compared to disease control group. There were significant increase in parameters related to oxidative stress like SOD, catalase and significant decrease in lipid peroxidation (MDA) and parameters related to inflammation like MPO level in animals treated with

EEFC (400 mg/kg) decreased as compared to disease control group. In present study all these parameters might be responsible for protective effect of Ethanolic extract of *Fagonia cretica* L. plant in DSS induced ulcerative colitis in mice. Thus further examination is expected to inspect the impact of each separated compound present in the plant extract and to explain the ultimate mechanisms of it's therapeutic mode.

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