Int J Pharm Bio Sci 2015 July ; 6(3): (P) 117 - 125

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

POTENTIAL OF *OXALIS CORNICULATA* LINN IN THE TREATMENT OF ULCERATIVE COLITIS

SANTOSH B. DIGHE [1]*, B. S. KUCHEKAR [2] AND SAGAR B. WANKHEDE[3]

[1]Department of Pharmacology, Pravara Rural College of Pharmacy, Pravranagar, Maharashtra, (India)
[2]Dept. of Pharmaceutical Chemistry, MAEER, Maharashtra Institute of Pharmacy, Pune Maharashtra, (India)
[3]Department of Pharmaceutical Chemistry, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, Maharashtra, (India)

ABSTRACT

Oxalis corniculata Linn (Oxalidace) is an Indian medicinal plant which is widely used as food and folk medicine. Present work was aimed to check the potential of *Oxalis corniculata* Linn leaf extract in the treatment of ulcerative colitis. Dried and powdered leaves were extracted using petroleum ether, chloroform, ethyl acetate and methanol. The extracts at a dose of 100 mg/kg were screened for acetic acid induced ulcerative colitis in mice. The antioxidant potential of extracts was evaluated by DPPH method. It was observed that liver and kidney functions were not affected in mice after administration of ethyl acetate and methanol extract for fifteen days. Treatment with ethyl acetate extract of *Oxalis corniculata* Linn showed superior antiulcer, antioxidant activity and better protective effect compared to other extracts. Myeloperoxidas and malondialdehyde level in blood and tissue was decreased significantly after administration of ethyl acetate and methanol extracts in mice.

KEYWORDS: ***Oxalis corniculata*** **Linn. Ulcerative colitis, Myeloperoxidase, Malondialdehyde,**

SANTOSH B. DIGHE
Department of Pharmacology, Pravara Rural College of Pharmacy, Pravranagar, Maharashtra, (India)

***Corresponding author**

INTRODUCTION

Oxalis corniculata Linn, Oxalidaceae, a sub-tropical plant is native of India, commonly known as changeri in marathi. This plant is a delicate-appearing, low growing, herbaceous and abundantly distributed in damp shady places and lawns nearly all regions throughout warmer part of Maharashtra.[1] It is locally used in treating various ailments. It is rich in niacin, vitamin C and β-carotene.[2] The juice of the plant is given in jaundice and in stomach troubles.[3] The leaf extract of the plant mixed with butter is applied to muscular swellings and pimples.[4] *Oxalis corniculata* Linn is also used as antiseptic, refrigerant, diaphoretic, diuretic and anti diabetic.[5] It is used as complementary medicine in wound healing, anemia, dyspepsia, cancer, piles, dementia and convulsions.[6,7] Other alternative uses are; anthelminthic, anti-inflammatory, astringent, depurative, diuretic, stomachic and styptic. It is also used in the treatment of urinary tract infections, influenza, fever, traumatic injuries and sprains.[8] It was also reported that plant has hypoglycemic, antipsychotic, nervous system stimulant and have chronotropic and inotropic effect.[9] Chemical characterization showed the presence of glyoxylic acid, oxalic acid, pyruvic acid, vitexin and isovitexin, vitexin-2-O-beta-D-glucopyranoside, neutral lipids, glycolipids; vitamin C; phospholipids; fatty acids, 18:2, 18:3, 16:0; saturated (C10-C14) acids; alpha and beta tocopherols. Ulcerative colitis (UC) is a subcategory of inflammatory bowel disease (IBD). Colitis affects the innermost lining or mucosa of the colon and rectum. A continuous area of inflammation and ulceration with no segments of normal tissue is observed. The two primary types of IBD are Crohn's disease and UC. In IBD, the intestine (bowel) becomes inflamed, often causing recurring abdominal cramps and diarrhea.[10] Although the exact cause of UC remains unknown, the condition appears to be related to a combination of genetic and environmental factors. Present study was designed to evaluate potential of various leaf extract of *Oxalis Corniculata* Linn in the treatment of acetic acid induced ulcerative colitis.

MATERIALS AND METHODS

Plant material

Leaves of *Oxalis corniculata* Linn were collected from the Loni (Shirdi) periphery in the Ahmednagar district of Maharashtra. It was authenticated by Dr. Diwakar, Joint Director of Botanical Survey of India, Pune. A voucher specimen (DSOC001) has been deposited in the herbarium section of the department of Pharmacognosy, PRCOP, Loni, for future reference. Collected leaves (1.5 kg) were air dried and pulverized using a mechanical grinder.

Animals

Male Swiss albino mice (20-25 g) were used. The animals were housed under standard laboratory conditions and fed with standard rodent diet and water *ad libitum*. Rodent diet is composed of crude proteins 16%, crude fats 3.8%, crude fibers 2%, amino acids, vitamins and minerals. The animals were kept in light-dark condition (12/12 h light/dark), temperature (22 ± 2°C) and humidity (55%). The experimental protocol was approved by institutional animal ethical committee (Approval No. 448/01/C/CPCSEA/14-15/18).

Extraction

Dried and powdered leaves (250 g) were extracted successively with various solvents viz. petroleum ether, chloroform, ethyl acetate and methanol in soxhlet extractor. The mark left was extracted using distilled water as solvent. Extracts were concentrated by vacuum distillation and then dried in open air to produce the respective extracts. All the extracts were vacuum dried to obtain the petroleum ether extract (7.24%), chloroform extract (6.034%), ethyl acetate extract (4.48%) and methanol extract (8.54%) respectively.

Pharmacological screening

i) Induction of experimental colitis

Animals were divided into eight groups. Control group mice received only vehicle (10% tween 80 in distilled water). Second group served as a standard which received prednisolone (5 mg/kg,

i.p.). The dose for screening was selected 100 mg/kg of all extract as per literature surveyand toxicity studies done at the plant. The animals of the third to sixth groups were treated with extract of petroleum ether (PEOC), chloroform (CFOC), ethyl acetate (EAOC) and methanol (MEOC) (100 mg/kg, i.p., each), respectively for 7 days. On 8th day colitis was induced by intrarectal administration of 150 µl of 5% acetic acid (pH 2.5), 3 cm from the anal margin. Extract treatment was continued up to 10th day.[11]

ii) Determination of ulcer index

The whole alimentary canal was isolated and colon was opened longitudinally and rinsed with phosphate buffer saline (PBS). The ulcer score of the opened colon was measured with the help of microscope and the ulcer index was calculated by following formula.[12]

$$\text{Ulcer index} = \frac{\text{Grade of ulcer in positive control} - \text{Grades of ulcers in test}}{\text{Grades of ulcer in test - Grades of ulcer in normal control}} \times 100$$

iii)Assessment of colitis severity

After 48 h of colitis induction mice were sacrificed by cervical dislocation and dissected upon to remove colon. The whole alimentary canal was isolated and colon was opened longitudinally and rinsed with phosphate buffer saline (PBS). Ulcer scoring of the colon damage was performed. For each mouse, the ulcer area was determined by summing the sizes of lesions measured macroscopically. The total area of damage was expressed as the relative percentage of the total surface area of the colon.[13]

iv) Determination of Myeloperoxidase activity (MPO) in colon and blood

After the macroscopic measurements, the excised colons (100-150 mg) were homogenized with PBS (pH 7.4) and centrifuged at 1000 rpm for 20 min at 4°C. Myeloperoxidase activity of supernatants was then assayed by mixing the supernatant with citric phosphate buffer (pH 5.0) containing 0.4 mg/ml O-phenylene diamine LR and 0.015% hydrogen peroxide. The change in absorbance at 492 nm was measured spectrophotometrically and compared with the standard dilution with horseradish peroxidase.[14]

v) Determination of Malondialdehyde level (MDA) in colon and blood

The reaction mixture containing 0.1 ml tissue sample, 0.2 ml 8.1% sodium dodecyl sulphate (SDS), 1.5 ml 2% acetic acid and 1.5 ml 0.8% aqueous solution of thiobarbituric acid. The mixture pH was adjusted to 3.5 and the volume was finally made up to 4 ml with distilled water and 5 ml of mixture of n-butanol and pyridine (15%) was added. The mixture was shaken vigorously. Centrifugation was performed at 4000 rpm for 10 min and absorbance of organic layer was measured at 532 nm. Malondialdehyde was expressed as n mol/mg of protein.[15]

vi) Histopathological study

Tissue was fixed with 10% formalin for 24-36 h and then trimmed at suitable site and washed under running tap water for 2 h then the tissue is dehydrated with help of increasing grades of alcohol (50% alcohol overnight, 70% alcohol for 2 h, 80% alcohol for 2 h, 90% alcohol for 2 h and absolute alcohol for 2 h). Then tissue was cleaned with xylene for 1 h and embedded with paraffin wax at 60°C. Blocks were prepared and stored in freezer for 45 days. Slices of tissue were cut at 5 mm thickness. Slices were taken on the clean grease free glass slides smeared with egg albumin in water bath at 60°C. Tissue was deparafinnated partially with heat and followed by immersing in the xylene for 3 min in each (3 changes of 3 min each). Sections rehydrated with decreasing grades of the alcohol [100, 90, 80, and 50% (3 min in each)]. Slides were kept in distilled water (5 min) and in hematoxyline (10 min). One dip was given in 1% ammonia water and immediately washed under running tap water (5 min). 2-3 drops of alcoholic

eosin were given and shades again dehydrated with increasing grades of alcohol [70, 80, 90, and 100% (3 min in each)]. Slices were cleaned with xylene, mounted with DPX mountant and observed under suitable magnification.[16]

vii) Evaluation of the antioxidant effect (in vitro)

The activity of 1,1-diphenyl,2-picrylhydrazyl (DPPH) radical scavenging activity was investigated according to the method described by Sreejayan and Rao.[15] The extracts i.e. Petroleum ether (PEOC), Chloroform (CFOC), Ethyl acetate (EAOC) and Methanol (MEOC) were tested at concentrations of 2, 4, 6, 8 and 10 mg/ml respectively. The absorbance of all mixtures of extracts and DPPH was recorded at 517 nm, with ascorbic acid (100 mM) used as standard. The data were expressed as an average percentage radical scavenging activity.

Percentage radical scavenging activity = [(Ac – At) / Ac] X 100
Where Ac and At are the absorbance of control (DPPH) and the test extract, respectively.[16]

viii)Effect on liver and kidney functions

Swiss albino mice of 20-25 gm body weight were divided into two equal groups each of six mice. The first group was left as a control, while the second group was given the ethyl acetate and methanol combined extracts orally in a dose (100 mg/kg) for fifteen days. Blood samples were collected from the orbital plexus of the mice, 6 h after medication. Samples were left to clot at room temperature for 20 min. The obtained sera were collected and used to determine the activity of aspirate aminotransferase and alanine aminotransferase.[17] Levels of urea and creatinine were also estimated.[18, 19, 20]

ix)Statistical analysis

All data were expressed as mean ± SEM. The statistical analysis of all the observations was carried out using one-way ANOVA followed by a multiple comparison test of Tukey–Kramer, where necessary. $P<0.05$ was considered as significant compared with the control group.

RESULTS AND DISCUSSION

Histopathological observation showed ulceration, hyperemia, necrosis, edema, cellular infiltration and goblet cell hyperplasia in the colon of mice treated with acetic acid. Treatment with ethyl acetate extract of *Oxalis Corniculata* Linn (EAOC) showed least ulceration and necrosis (Table 1, Figure 2). EAOC and MEOC showed a better protective effect in ulcer index than other extract comparable with the standard drug (Table 2). Myeloperoxidase level was increased up to 347 U/ml and 370 U/mg respectively in blood and tissue after administration of acetic acid. After treatment with EAOC at a dose of 100 mg/kg myeloperoxidase level in blood and tissue was decreased up to 250 U/ml and 259 U/mg while MEOC at 100 mg/kg dose was found 293 U/ml and 302 U/mg. Acetic acid caused an increase in malondialdehyde level in blood and tissue up to 12.33 nmol/ml and 14.74 nmol/mg, respectively. After treatment with EAOC at a dose of 100 mg/kg malondialdehyde level in blood and tissue was decreased up to 8.77 nmol/ml and 9.14 nmol/mg while MEOC at 100 mg/kg dose was 8.89 nmol /ml and 9.21 nmol /mg. (Table 3) The extracts of *Oxalis corniculata* Linn showed activity towards scavenging of the DPPH radical in all extract. The activity of the ethyl acetate extract was the highest and ranged from 27.51% for the lower concentration to 84.50% for the largest one, compared to 87.8% for the standard ascorbic acid at a concentration of 100 mM. The petroleum ether extract showed the lowest activity among the extracts (Table 4 and Fig. 1). Also both liver and kidney functions were not affected after combine administration of ethyl acetate and methanol extract. There was no significant difference between the control and test groups in the experiments, at the 0.05 level of probability (Table 5). Significant reduction was observed with myeloperoxidase and malondialdehyde levels after administration of ethyl acetate and methanol extract. As EAOC and MEOC reduced myeloperoxidase and malondialdehyde levels significantly, it may have potential anti-inflammatory role in the treatment of colitis because myeloperoxidase is involved in the inflammatory reaction in colitis [21] and

increased level of malondialdehyde is indicating oxidative stress in organ and thus causing inflammation.[22] Cytokines are responsible for modulating intestinal inflammation and injury.[23, 24] Increased levels of TNF-α and PGE2 may cause epithelial cell necrosis, edema, and neutrophil infiltration, as proved by the histopathological study. Recently it has been found that LITAF (lipopolysaccharide-induced TNF-α factor), which mediates TNF-α expression in human macrophages, is significantly elevated above controls in macrophages of ileal and colonic tissues from patients with ulcerative colitis.[25] In this study different extracts of *Oxalis corniculata* Linn decreased significantly the gross lesion scores, and may inhibit production of TNF-α and PGE_2. Inhibition of PGE2, on the other hand, may follow that of TNF-α or may result from its ability to inhibit cycloxygenase enzymes.[26, 27] Intestine is in a constant state of inflammation, therefore amplification of the inflammatory response activates infiltration of inflammatory cells that triggers pathological responses and symptoms of inflammatory bowel disease.[28] Our study showed that acetic acid raised the levels of colonic myeloperoxidase, which indicates infiltration of neutrophils and perturbation of the inflammatory system. This fact is documented in both animal models and patients with inflammatory bowel disease.[29] In ulcerative coloitis, oxidative stress plays a role in disease initiation and progression. Reactive oxygen species (ROS) attack the cellular macromolecules, thus disrupting epithelial cell integrity and hindering mucosal recovery, especially in case of impaired endogenous defense systems.[30] The antisecretory mechanism of extracts includes the antioxidant property. The DPPH radical is considered to be model of a stable lipophilic radical a chain reaction in lipophilic radicals was initiated by the lipid autooxidation, antioxidant molecules react with DPPH reducing a number of DPPH molecules equal to number of their hydroxyl groups. Therefore, the absorption at 517 nm was proportional to the amount of residual DPPH.[31, 32] Hence antioxidants play a major role in repairing the ulcerative colitis.[33]

Table 1
Histopathological observations after the treatment with extracts of Oxalis Corniculata Linn.

Treatment	Ulceration	Hyperemia	Necrosis	Edema	cellularin filtration	Gobletcell hyperplasia
Normal	00	00	00	00	00	+
Control (5% Acetic acid)	++++	++++	++++	++	+++	+++
Prednisolone (5 mg/kg)	++	++	++	++	++	++
PEOC (100 mg/kg)	+++	+++	+++	++	+++	+++
CFOC (100 mg/kg)	+++	++	+++	++	+++	++
EAOC (100 mg/kg)	++	++	++	++	+++	++
MEOC (100 mg/kg)	++	++	++	++	++	++

0: no abnormality detected
+: damage/ active changes up to less than 25%
++: damage/ active changes up to less than 50%
+++: damage/ active changes up to less 75%
++++: damage/ active changes up to more than 75%

Table 2
Effect of different extracts of Oxalis Corniculata Linn in acetic acid induced ulcerative colitis

Treatment	Ulcer index	Ulcer protection (%)
Normal	-	100
Control (5% Acetic acid)	117.6 ± 13.47	0
Prednisolone (5 mg/kg)	16.7 ± 2.78***	60
PEOC (100 mg/kg)	63.3 ± 6.59	10
CFOC (100 mg/kg)	57.4 ± 8.81*	20
EAOC (100 mg/kg)	23.3 ± 1.97**	30
MEOC (100 mg/kg)	44.9 ± 4.30**	40

Values are mean ± S.E.M. *p<0.0001 extremely significant, **P< 0.01 very significant, ***<0.05 significant as compared to control group.

Table 3
Effect of extracts of Oxalis Corniculata Linn on Myeloperoxidase and Malondialdehyde level.

Treatment	Myeloperoxidase		Malondialdehyde	
	Blood (U/ml)	Tissue (U/mg)	Blood nmol/ml	Tissue nmol/mg
Normal	94 ±1.1	102 ±1.2	3.88 ± 0.9	4.78 ± 1.3
Control (5% Acetic acid)	347±1.4	370 ±1.3	12.33 ± 0.8	14.74 ± 1.3
Prednisolone (5 mg/kg)	304±1.5*	310 ±1.9*	9.58 ± 1.3*	10.44 ±1.5*
PEOC (100 mg/kg)	304±0.9	353 ±1.8	9.89 ± 1.8	11.52 ± 0.9
CFOC (100 mg/kg)	316±1.2	326 ± 1.2	8.79 ± 0.9*	10.41 ± 0.8*
EAOC (100 mg/kg)	250±1.9*	259 ± 1.3*	8.77 ± 1.8*	9.14 ± 1.2*
MEOC (100 mg/kg)	293±1.7*	302 ± 1.2*	8.98 ± 1.5*	9.21 ± 0.9*

Values are mean ± S.E.M. *p<0.0001 extremely significant, **P< 0.01 very significant, ***<0.05 significant as compared to control group.

Table 4
Effect of extracts and isolated compounds from O. Corniculata Linn. on scavenging DPPH radical

Radical scavenging activity (%)					
Extract	**2 (mg/ml)**	**4 (mg/ml)**	**6 (mg/ml)**	**8 (mg/ml)**	**10 (mg/ml)**
Petroleum ether (PEOC)	11.29 ± 1.00	16.38 ± 1.09	18.19 ± 0.99	25.95 ± 1.13	30.60 ± 1.07
Chloroform (CFOC)	15.22 ± 1.01	21.35 ± 0.98	34.50 ± 1.12	46.88 ± 1.04	50.98 ± 0.95
Ethyl acetate (EAOC)	27.51 ± 1.01	38.94 ± 0.98	53.63 ± 0.99	68.72 ± 1.12	84.50 ± 0.99
Methanol (MEOC)	28.65 ± 1.01	33.01 ± 0.93	48.35 ± 1.01	54.33 ± 0.88	64.90 ± 0.79

Values are mean ± S.E.M. *p<0.0001 extremely significant, **P< 0.01 very significant, ***<0.05 significant as compared to control grou

Table 5
Effect of 15 days daily administrations of combined ethyl acetate and methanol extract on kidney and liver functions

	Liver functions		Kidney functions	
Treatment	ALT (U/l)	AST (U/l)	Urea (mg/dL)	Creatinine (mg/dL)
Control	12.80 ± 0.59	15.00 ± 0.71	52.82 ± 3.57	0.97 ± 0.017
EAOC + MEOC	13.60 ± 3.99	15.80 ± 1.02	68.56 ± 5.21	1.078 ± 0.05

Values are mean ± S.E.M. *p<0.0001 extremely significant, **P< 0.01 very significant, ***<0.05 significant as compared to control group.

Figure 1

Radical scavenging activity of the extracts of Oxalis Corniculata linn. at concentrations 2, 4, 6, 8 and 10 mg/ml.

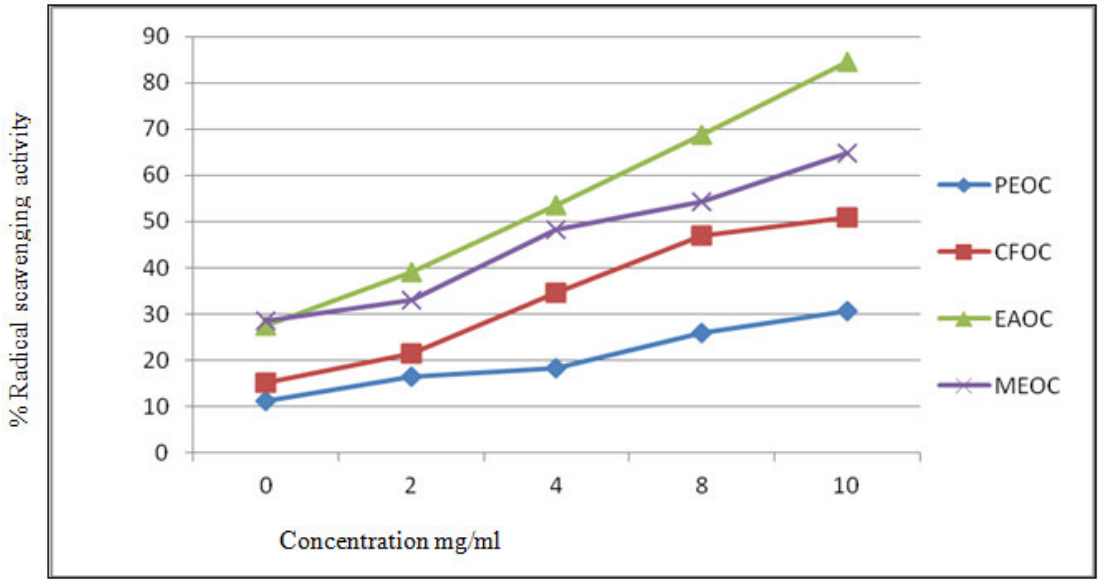
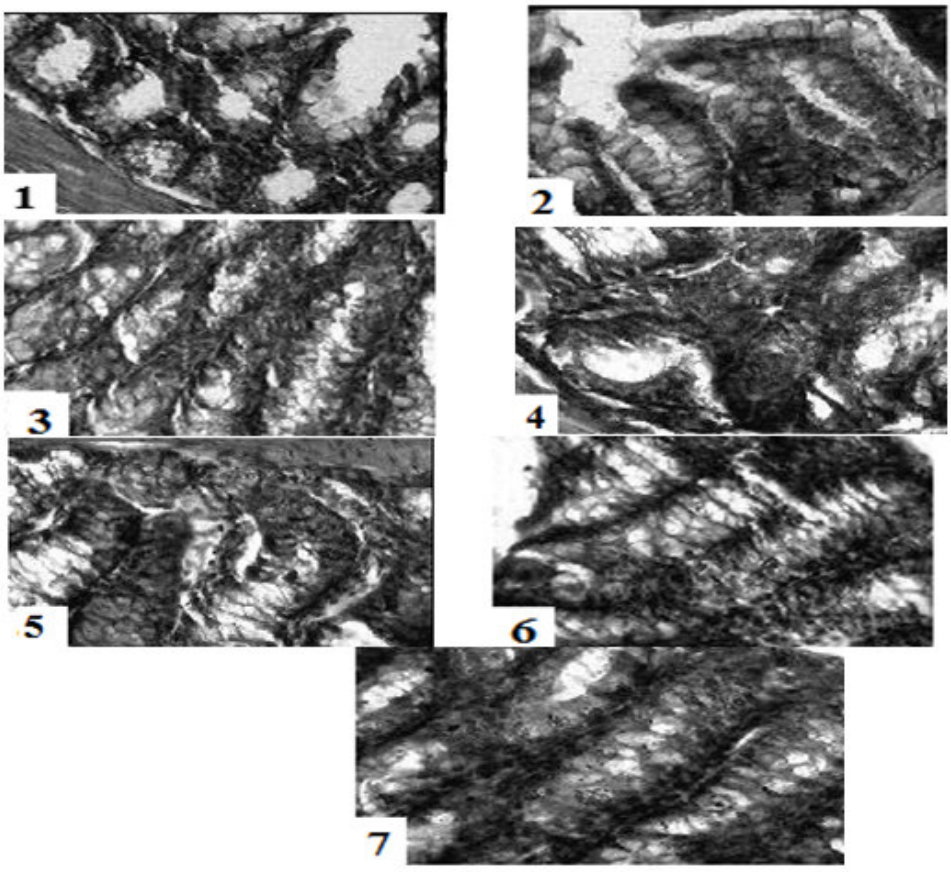


Figure 2

Histopathological results of colon tissue after the treatment of different extracts of Oxalis corniculata Linn in acetic acid induced ulcerative colitis. 1 = Normal tissue; 2 = Negative control (5% acetic acid); 3 = Standard (Prednisolone, 5 mg/kg), 4 = PEOC (100 mg/kg), 5= CFOC (100 mg/kg), 6= EAOC (100 mg/kg), 7= MEOC (100 mg/kg)



CONCLUSION

In this work, acetic acid induced ulcerative colitis formation is inhibited by ethyl acetate and methanol extract of *Oxalis Corniculata* Linn more significantly. The mechanism is believed to operate by inhibiting endogenous prostaglandin levels, decreasing histamine secretion and scavenging oxygen derived free radicals. Hence study confirmed the traditional uses of leaves of *Oxalis corniculata* Linn in treatment of ulcerative colitis.

REFERENCES

1. Kirtikar K.R. and Basu. Indian Medicinal Plants, 3rd ed. New Delhi: MS periodical experts; 1: 437, (1975).
2. Manandhar N. P., Plants and People of Nepal, Portland, Oregon: Timber Press, 599, (2002).
3. Hussain K., Shahazad A., Hussnain Z. U. An ethnobotanical survey of important wild medicinal plants of Hattar, District Haripur, Pakistan, Ethnobotanical Leaflets, 12: 29-35, (2008).
4. Nadkarni K.M. Indian Materia Medica, 1st Edn, Vol 3, Bombay Popular Prakashan Pvt. Ltd: Bombay, 115–116, (1982).
5. Sharma R. A., Kumari A. Phytochemistry, pharmacology and therapeutic application of *oxalis corniculata* linn. A review, int j pharm pharm sci, 6(3): 6-12, (2014).
6. Taranalli A.D., Tipare S.V., Kumar S., Torgal S. S. Wound healing activity of Oxalis corniculata whole plant extract in rats, Ind. J. Pharma. Sci, 66 (4): 444-446, (2004).
7. Madhavachetty K., Sivaj J. K., Tulasi R. K. Flowering plants of Chittoor district, Andhra Pradesh, India, 1st ed. Student offset printers, Tirupati : 54-55, (2008).
8. Achola K. J., Mwangi J. W., Munenge R. W., Pharmacological activity of Oxalis corniculata, Pharm. Biol. 33: 247-249, (1995).
9. Raghavendra M. P., Satish S., Raveesha A. Phytochemical analysis and antibacterial activity of Oxalis corniculata, a known medicinal plant, My Science 1: 72-78, (2006).
10. Badmapriya D., Sathish K. V. Profile Of Ulcerative Colitis In South Indian Region: Karaikal, Int J Pharm Bio Sci, 2(1): 47-51, (2011).
11. Ganjare A. B., Nirmal S. A., Rub R. A., Patil A. N., Pattan S. R. Use of *Cordia dichotoma* bark in the treatment of ulcerative colitis. Pharmaceutical Biology, 49(8): 850-855 (2011).
12. Nirmal S. A., Ingale J. M., Pattan S. R., Bhawar S. B. *Amaranthus roxburghianus* root extract in combination with piperine as a potential treatment of ulcerative colitis in mice. Journal of Integrative Medicine, 11(3): 206-212 (2013).
13. Gholap P. A., Nirmal S. A., Pattan S. R., Pal S. C., Mandal S. C. Potential of *Moringa oleifera* root and *Citrus sinensis* fruit rind extracts in the treatment of ulcerative colitis in mice. Pharmaceutical Biology, 50(10): 1297-1302 (2012)
14. Zaware B. B., Nirmal S. A., Baheti D. G., Patil A. N., Mandal S. C. Potential of *Vitex negundo* roots in the treatment of ulcerative colitis in mice. Pharmaceutical Biology, 49(8): 874-878 (2011).
15. Ganjare A. B., Nirmal S. A., Patil A. N. Use of apigenin from *Cordia dichotoma* in the treatment of colitis. Fitoterapia.82: 1052-1056, (2011).
16. Nirmal S. A., Dhikale R. S., Girme A. S., Pal S. C., Mandal S. C. Potential of the plant *Thespesia populnea* in the treatment of ulcerative colitis. Pharm Biol, 4(10) :1-7, (2015).
17. Reitman S., Frankel S., Glutamic oxalo-acetic and glutamic pyruvic transaminases. Am J Pathol, 28: 56–63, (1957).
18. Fawecett J., K., Scott J.E., Enzymatic determination of urea. J Clin Pathol, 13: 156–59, (1960).

19. Patton G.J., Crouch S. R. Colorimetric method for the determination of serum urea. Anal Chem, 49: 464–9,(1977).
20. Husdan H., Rapoport A., Estimation of creatinine by the Jaffe reaction. Clin Chem, 14: 222–238, (1968).
21. Cetinkaya A., Bulbuloglu E., Kurutas E. B., Ciralik H. Kantarceken B., Buyukbese M. A. Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats. Tohoku J Exp Med, 206: 131-139, (2005).
22. Church D. F., Pryor W. A., Free radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect, 64: 111-126, (1985).
23. Ardizzone S., Bianchi P. G., Biologic therapy for inflammatory bowel disease. Drugs, 65: 2253-2286, (2005).
24. Nakamura K., Honda K., Mizutani T., Akiho H., Harada N. Novel strategies for the treatment of inflammatory bowel disease: Selective inhibition of cytokines and adhesion molecules. World J Gastroenterol, 12: 4628-4635, (2006).
25. Stucchi A., Reed K., O'Brien M., Cerda S., Andrews C., Gower A., Bushell K., Amar S., Leeman S., Becker J. A new transcription factor that regulates TNF alpha gene expression, LITAF, is increased in intestinal tissues from patients with CD and UC. Inflammatory Bowel Disease, 12: 581–587, (2006).
26. Otani T., Yamaguchi K., Scherl E., Du B., Tai H., Greifer M., Petrovic .L, Daikoku T., Dey S., K., Subbaramaiah K., Dannenberg A. J. Levels of NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase are reduced in inflammatory bowel disease: evidence for involvement of TNF-α. American Journal of Physiology, Gastrointestinal and Liver Physiology, 290: 361– 368, (2006).
27. Sartor R. B. Pathogenesis and immune mechanisms of chronic inflammatory bowel disease. The American Journal of Gastroenterology, 92: 5S–11S, (1997).
28. Akgun E., Caliskan C., Celik H. A., Ozutemiz A. O., Tuncyurek M., Aydin H. H., Effects of *N*-acetylcysteine treatment on oxidative stress in acetic acid induced experimental colitis in rats. J Int Med Res, 33: 196-206,(2005).
29. Kruidenier L., Kuiper I., Lamers C., Verspaget H. W. Intestinal oxidative damage in inflammatory bowel disease: Semi-quantification, localization, and association with mucosal antioxidants. J Pathol, 201: 28-36,(2003).
30. Buffinton G. D., Doe W. F. Depleted mucosal antioxidant defenses in inflammatory bowel disease. Free Radic Biol Med, 19: 911–918, (1995).
31. Lastra L. A., Martin M. J., Motilva V. Antiulcer and gastroprotective effects of quercetin: A gross and histologic study. Pharmacol, 48: 56–62, (1994).
32. Martin M. J., La C. C., Alarcon de la Lastra C., Cabeza J., Villegas I., Motilva V. Anti-oxidant mechanisms involved in gastroprotective effects of quercetin Z Naturforsch C, 53: 82–88, (1998).
33. Olaleye S. B., Farombi E. O., Attenuation of indomethacin and HCl ethanol-induced oxidative gastric mucosa damage in rats by kolaviron, a natural biflavonoid of Garcinia kola seed. Phytother Res 20: 14–20, (2006).