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Research Article

# Evaluation of Ethanolic Leaf Extract of *Cucumis melo* var. agrestis in Acetic Acid-induced Ulcerative Colitis on Rats

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ABSTRACT

The crux of the present research work was to evaluate the therapeutic potential of Ethanolic leaf extract of *Cucumis melo var. agrestis* (ELECM) on Ulcerative Colitis in rats. The successful induction of Ulcerative Colitis was done by intra-rectal administration of 500 µl of acetic acid (4% v/v), rats were euthanized on day 8 by cervical dislocation under anesthesia. The pathological parameters such as colon weight, colon antioxidant enzymes (SOD, CAT), lipid peroxidase enzymes (MDA, MPO), inflammatory markers (TNF- α and IL-6), and histopathological aspects were estimated to evaluate the ELECM against the disease control. Sulfasalazine was used as positive control to compare the protective effect of the ELECM. The GC-MS analysis of ELECM confirms the presence of about 12 specific phytochemicals. Induction of ulcerative colitis was evidenced in the control group due to increased colon weight, lipid peroxidase enzymes, and immune markers also by alleviated levels of antioxidant enzymes in the rat colon. However, ELECM treated group almost reversed the effect of the disease control group, above all the histopathological response of the ELECM-treated group against the acetic acid-induced ulcerative colitis was almost the same as that of the control group, indicating the significant protective effect. Our present study suggests that ELECM extract has significant protective activity against acetic acid-induced ulcerative colitis, due to its higher antioxidant, moderate anti-inflammatory, and mild immune-suppressive effects, and the protective action might be due to the presence of active constituents such as flavonoids and total phenols as presented on the GC-MS analysis report.

## Introduction

Ulcerative colitis is a chronic inflammatory bowel disease with recurrence, symptomized by nausea, diarrhea, loss of weight, and abdominal pain with a severe impact on quality of life.[1] The etiological backroad is not clear. However, it has a genetic, immunological, and environmental link.[2] Pathologically reactive oxygen species and reactive nitrogen species with depletion of antioxidants[3] cause extensive mucosal damage and facilitate the disease's progression. The damaged epithelial cells consciously undergo the healing process by restitution, proliferation, and differentiation.[4,5] Acute colitis is devastated by marked inflammatory cell infiltration, activation, and downstream expression of pro-inflammatory mediators including TNF-α and interleukin 6 (IL-6). The pharmacotherapy of commonly used drugs such as 5-aminosalicylic acid, systemic corticosteroids, immune-modulators, and vitamin E [6] is challenged by immunity, instigation of microbes, and toxicities.[7,8] The phytochemical-based treatment might be a better, more effective alternative and free from an adverse drug reaction.

We chose acetic-acid-induced ulcerative colitis in a rat model for our current research, as this model was found to be simple, less time-consuming, and more relevant to the pathology of human ulcerative colitis. It is also useful for the screening of antiulcer drugs.[9] The fruit of

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*Cucumis melo var. agrestis,* popularly known as Wild Melon also known as musk melon is an annual creeper widely consumed vegetable distributed in most parts of India.[10] The ethanolic leaf, seed, and fruit extract contains rich phytochemicals such as triterpenes flavonoids, phenolic compounds, steroids, alkaloids, essential oil, and tannins.[11] Musk melon is one of the valuable medicinal plants against pain, immunity, free radicals scavenging, and inflammatory management,[12-16] in addition to that it also possesses diuretic,[17] anticancer,[18,19] antioxidant[20] antiulcer,[21] antidiabetic,[22,23] anti-fertility,[24] anti-thyroidal[25] anthelmintic and anti-bacterial[26] potential, besides no side effects and musk melon can be considered as distinctive, affordable, tasty and safe fruit with wide medicinal value. In *C. melo var. agrestis* the presence of multiple and valuable phytochemicals, especially the flavonoids and phenolic compounds, has shown to posses varieties of proven pharmacological activity among that the presence of anti-inflammatory effects, made us to select ELECM for the evaluation of IBS. The flavonoids as it dissolves more in 70% ethanol compared to other solvents due to its rich polarity we selected ELECM for our current research work.[27,28]

## Materials and Method

### Plant Collection and Extraction

Fresh leaves of *C. melo var argestis* were collected from the village of Thoothukudi region, Tamil Nadu, India in December 2020. Collected plant materials were air-dried, powdered by a mechanical mixer, and sieved under mesh sizes 40 and 60. The resultant 500 g of coarse powder was defatted with petroleum ether followed by continuous hot extraction with the help of the soxhlet apparatus using 70% ethanol. The greenish gummy extract obtained was freeze-dried. The final alcohol-free extract was found to be soluble in both water and alcohol, the percentage yield of moisture-free ELECM was 6.48 w/w.

### Phytochemical Analysis

In our study, GS-MS was employed to identify and correlate the phytochemical components present in the MEPC. It was carried out at Sitra laboratory, Coimbatore, Tamil Nadu. The GC-MS separates chemical mixtures, identifies each chemical at a molecular level in the most accurate manner, and is considered to be a definitive analytical tool. GS-MS uses inert gas (helium) to carry the separated chemicals that emerge from the column opening, when it flows into the MS it identifies the analyte molecule using a library of spectra with corresponding compounds stored in the computer.

### Animals

Ten weeks old healthy male wister rats obtained from an authorized source was quarantined and allowed to acquire standard laboratory condition for 2 weeks. All the animals were given a standard commercial diet and allowed to access the water *ad libitum*. The room temperature was maintained at 23 ± 2°C through air conditioning with 24 hours dark-light cycling throughout the study. The strict experiment protocol was followed as per CPCSEA guidelines and IAEC approval (KMCRET/ReRe/MPharm/11/2021) was obtained before the study. During the ulcerative colitis induction by intra-rectal acetic acid administration, the animals have exposed under 50 mg/kg pentobarbital *(i.p.*) anesthesia.

### Induction of Ulcerative Colitis

Randomly allotted animals were divided into 5 groups (n = 6), all groups received the vehicle, standard, and test drug with the test dose of 200 and 400 mg/kg for low and high dose of ELECM based on literature review[29] and dose is based on body weight as per the group illustrated in Table 1 for 1 to 7 days,[30] with the intervention of 500 µL of 4% (v/v) acetic acid by intra-venous cannula (21G) by intra-rectal route (6–7 cm from the anus) under 50 mg/kg pentobarbitone *(i.p.*) anesthesia, after 2 hours administration of vehicle, standard and test drug for the respective groups except the control group on 4$^{th}$ day. On the 8$^{th}$ day, all group animals were killed under anesthesia, the caecum and distal part of the colon was removed for macroscopical and microscopical assessment, and further, the colon tissues were homogenized for biochemical parameters estimation.

### Estimation of Rat Colon Weight

All groups of rat colons were dissected by opening the colon longitudinally, rinsed with tap water to remove the feces then the cleaned colon was placed on a clean surface. The equal length of all group colons was weighed and tabulated.

### Estimation of Antioxidant Enzyme Activity in Colon

The antioxidant enzyme levels such as superoxide dismutase (SOD) and catalase (CAT) were estimated by the homogenized colon samples on ice-cold tris buffer (0.02 M pH of 7.4) to get a 10% of homogenate sample.

#### *Estimation of SOD levels in colon*

The estimation was done based on the spectrophotometric principle[31] by adding 750 µL ethanol (96% v/v) and 150 µL of ice-cold chloroform into 500 µL of the colon homogenate then it was centrifuged at 2000 rpm for 20 minutes at 25°C from that 500 µL supernatant was collected additional 500 µL EDTA (0.6 mM) and 1-mL carbonate bicarbonate buffer (0.1 M, pH 10.2) were added. By the addition of 50 µL of adrenaline (1.3 mM), The reaction was initiated and the absorbance was recorded at 480 nm. Absorbance was measured against a blank at 480 nm using a UV spectrophotometer.

The following formula calculated the percentage inhibition of autoxidation of adrenaline and the amount of enzyme inhibited by auto-oxidation of adrenaline by 50% at 25°C.

Percentage inhibition = Σ Absorbance test – Absorbance blank Σ× 100:ð1Þ

The SOD activity (Unit of SOD activity/mg protein = Σ % inhibition Σ× 100

*Estimation of catalase levels in colon*

The spectrophotometric estimation of catalase was done[32] by the addition of 100 µL of the supernatant colon tissue homogenate with a mixture containing 1-mL (0.01 M) phosphate buffer (pH 7.0), 500 µL H2O2 (1.18 M), and 400 µL of water and the reaction is initiated by incubating the sample at 28°C for 5 minutes, the reaction was terminated using a mixture comprising a 3: 1 ratio of glacial acetic acid and 5% potassium dichromate resulting in the formation of chromic acetate which is measured 620 nm by UV spectrophotometer. The enzyme activity was expressed in terms of its molar extinction coefficient of 39.4 $M^{-1}$ $cm^{-1}$.

## Estimation of Lipid Peroxidase Enzyme levels in Colon

*Estimation of malondialdehyde (MDA) levels in colon*

The elevation of MDA denotes the excess amount of lipid peroxidation process occurs during the pathology of ulcerative colitis by acetic acid induction, estimated by spectrophotometric principle[33] using the tissue homogenate of the colon of about 1-mL with the addition of 3 mL of a mixture comprising of 20% trichloroacetic acid (TCA) and 0.5% thiobarbituric acid (TBA). The resultant sample was heated at 95°C for 30 minutes, cooled in an ice bath, and centrifuged at 2,000 rpm for 10 minutes. The absorbance of the MDA-TBA complex was measured at 532 nm against the blank using a UV spectrophotometer. MDA's concentration (nmol/mg protein) was calculated using the MDA extinction coefficient of 1:56 × 10-5 $M^{-1}$ $cm^{-1}$.

*Estimation of myeloperoxidase (MPO) levels in Colon*

The estimation of MPO indirectly denotes the neutrophil content of the colon tissue as the enzyme was present within the granules of neutrophils.[34] The entire procedure of MPO levels was estimated under dark conditions using a 96-well plate with a microplate reader at 490 nm. 50 µL of the sample was taken in duplicate. To this, 250 µL of ODA-$H_2O_2$ was added, comprising 680.45 mg of potassium dihydrogen orthophosphate in 100 mL of distilled water and the pH was adjusted to 6.0. ODA solution includes 0.167 mg of ODA in 1 mL of phosphate buffer of pH 6.0. Finally, ODA-$H_2O_2$ was prepared by adding 1-mL of 30% of H2O2 to 1-mL of ODA solution. In addition, the reading was noted at 5 and 15 minutes. After this, 4 M $H_2SO_4$ was added to stop the reaction, and again the reading was noted. The concentrations of MPO at subsequent time intervals were determined from the standard plot which uses horse radish peroxidase as standard.

*Estimation of serum immune markers (TNF-α and IL-6) levels*

At the end of the experiment (day 8), all group rats were euthanized, blood samples were collected and centrifuged at 3000 rpm for 30 minutes at 4°C and the serum was obtained for the estimation of TNF-α and IL-6 using ELISA kits as per the manufacturer instruction.

## Microscopical Examination of Colon and Caecum

The caecum and a distal part of colons of all group rats were immediately fixed with 10% formaldehyde, embedded in liquid paraffin, about 5 µm thick transverse sections were cut and mounted on glass slides stained by hematoxylin and eosin (H & E) mixture. The acetic acid exposure damage to the colon and caecum was assessed and compared among the groups through the cellular damages mediated by infiltration, ulceration, hyperemia, necrosis, fibrosis, and submucosal abscesses.

## Data Analyses

The statistical method for all the data was analyzed by one-way ANOVA followed by Dunnet's multiple comparison test. All the statistical analysis was performed using GraphPad Prism software Differences in p-values < 0.05 were considered to be statistically significant for comparison purposes. The ensuing results were then presented as mean ± SEM.

# Results

## Phytochemical Analysis

The qualitative phytoconstituent analysis of ELECM by GS-MS and further comparing the chemical library showed the presence of about 12 specific phytochemicals as presented in Fig. 1.

## Rat Colon Weight

The colon weight of the acetic acid-induced ulcerative colitis rats (1.37 ± 0.03 g) was observed which is almost a two-fold increase as compared to the control group (0.73 ± 0.03 g). However, the standard (1.01 ± 0.04 g) and test low dose (1.18 ± 0.03 g) and high dose (1.07 ± 0.05 g) of ELECM have a substantial reduction in colon weight, indicating both prednisolone and ELECM have anti-inflammatory activity against ulcerative colitis induced by acetic acid (Table 2 and Fig. 2) in rats.

Chromatogram ASG 326- *Cucumis melovaragretis*D:\GCMS Data\ December 2021\15.12.2021\ASG 326- *Cucumis melovaragretis*. qg17,420,818

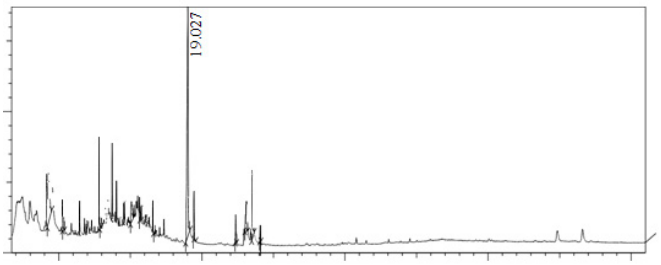


**Fig. 1:** ELECM of GC-MS Chromatogram

| *Peak#* | *R.Time* | *Area* | *Area%* | *Height* | *Height%* | *Name* | *Base m/z* |
|---|---|---|---|---|---|---|---|
| 1 | 9.258 | 29094883 | 13.60 | 3469036 | 7.39 | 5-Hydroxymethylfurfural | 97.05 |
| 2 | 10.311 | 6795426 | 3.18 | 2143756 | 4.57 | 2-Methoxy-4-vinylphenol | 150.05 |
| 3 | 12.905 | 12974529 | 6.06 | 6440325 | 13.72 | 1H-Inden-1-one, 2,3-dihydrotetramethyl- | 173.10 |
| 4 | 15.042 | 9639649 | 4.51 | 1219121 | 2.60 | Bergamotol, Z-.alpha.-trans- | 93.10 |
| 5 | 15.714 | 4502376 | 2.10 | 1634497 | 3.48 | Tetradecanoic acid | 73.05 |
| 6 | 16.675 | 4767699 | 2.23 | 2357174 | 5.02 | Neophytadiene | 68.10 |
| 7 | 19.027 | 89688666 | 41.92 | 16224633 | 34.57 | Hexadecanoic acid | 73.00 |
| 8 | 19.513 | 10733605 | 5.02 | 3465160 | 7.38 | Ethyl palmitate | 88.05 |
| 9 | 22.366 | 5955906 | 2.78 | 2003035 | 4.27 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 57.05 |
| 10 | 23.118 | 12706586 | 5.94 | 2131290 | 4.54 | Alpha.-Linolenic acid | 79.05 |
| 11 | 23.554 | 24026593 | 11.23 | 4709077 | 10.03 | 9-Octadecenoic acid (z)- | 55.05 |
| 12 | 24.084 | 3076828 | 1.44 | 1142024 | 2.43 | Ethyl stearate | 88.05 |

**Table-1:** Induction of acetic acid induced inflammatory bowel diseases on rats

| *S. No* | *Groups* | *Treatment* |
|---|---|---|
| 1 | Group-1 | Normal saline for 7 days |
| 2 | Group-2 | Normal saline for 7 days + *intra-rectal* administration of 500µL of acetic acid (4% v/v) after 2 hrs Normal saline (*p.o.*) on 4th day. |
| 3 | Group-3 | Standard (Prednisolone 10 mg/kg, *p.o.*) for 7 days + *intra-rectal* administration of 500µL of acetic acid (4% v/v) after 2 hrs prednisolone(*p.o.*) on 4th day. |
| 4 | Group-4 | ELECM (200 mg/kg, *p.o.*) for 7 days + *intra-rectal* administration of 500µL of acetic acid (4% v/v) after 2 hrs ELECM(*p.o.*) on 4th day. |
| 5 | Group-5 | ELECM (400 mg/kg, *p.o.*) for 7 days + *intra-rectal* administration of 500 µL of acetic acid (4% v/v) after 2 hrs ELECM(*p.o.*) on 4th day. |

**Table 2:** Effect of ELECM on colon weight and colon antioxidant activity

| *S. no* | *Groups* | *Colon weight (g)* | *Colon SOD level (U/mg of protein)* | *Colon CAT level (nmol /min/mg of protein)* |
|---|---|---|---|---|
| 1 | Control | 0.73 ± 0.04 | 8.90 ± 0.22 | 10.78 ± 0.22 |
| 2 | Negative | 1.37 ± 0.03 | 2.81 ± 0.16 | 3.43 ± 0.17 |
| 3 | Positive | 1.01 ± 0.04 | 6.48 ± 0.18 | 7.76 ± 0.25 |
| 4 | LD ELECM | 1.18 ± 0.03 | 6.22 ± 0.16 | 6.04 ± 0.10 |
| 5 | HD ELECM | 1.07 ± 0.05 | 5.63 ± 0.20 | 6.88 ± 0.17 |

Data are presented as mean ± SEM, n=6. Control, Negative control (Acetic acid induced), Positive Control (Prednisolone), Low Dose, High Dose of ELECM. One-way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * p<0.05, ** p<0.01, *** p<0.001

## Antioxidant Enzyme Activity on Colon

The reduced level of tissue antioxidant is one the evidence of induction of ulcerative colitis, in our study, there was about 68% reduction of colon tissue SOD in the disease control group [2.81 ± 0.16 (U/mg of protein)] as compared to the control group [8.90 ± 0.22 (U/mg of protein)], conform the induction of ulcerative colitis. However, there was a substantial increase (less %reduction) of 57, 55, and 50 on standard [6.48 ± 0.18 (U/mg of protein)] and low [6.22 ± 0.16 (U/mg of protein)] and high dose [5.63 ± 0.20 (U/mg of protein)] of ELECM treated rats were observed (Table 2 and Fig. 3).

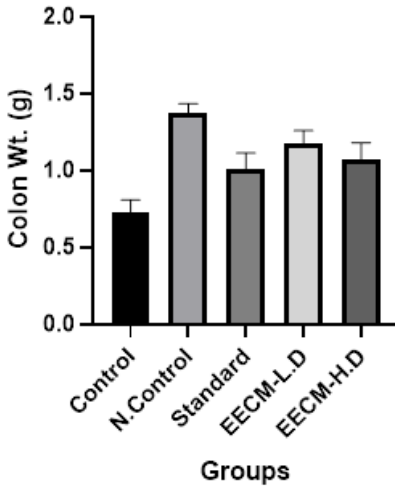


**Fig. 2:** Effect of ELECM on colon weight

Data are presented as mean ± SEM, n=6. Control, Negative control (Acetic acid induced), Positive Control (Prednisolone), Low Dose, High Dose of ELECM. One-way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * p<0.05, ** p<0.01, *** p<0.001

Similarly, there was about a 68% reduction of colon tissue Catalase in the ulcerative colitis control group [3.43 ± 0.25 (U/mg of protein)] as compared to the control group [10.78 ± 0.22 (U/mg of protein)], conform the induction of ulcerative colitis. However, there was a substantial increase (less %reduction) of 28, 44, and 36 on standard [7.76 ± 0.25 (U/mg of protein)] and low [6.03 ± 0.10 (U/mg of protein)] and high dose [6.88 ± 0.17 (U/mg of protein)] of test treated rats were observed (Table 2 and Fig. 4).

## Lipid Peroxidation

The significant alleviated tissue levels of MPO were observed in standard and test groups as 80.53 ± 2.96, 76.33 ± 2.66, and 62.66 ± 2.41 (µg/mg of tissue) as compared to

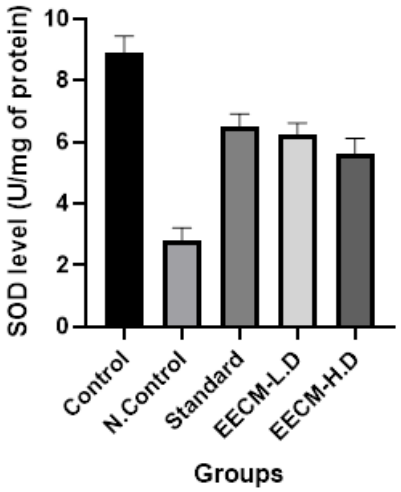


**Fig. 3:** Effect of ELECM on colon tissue SOD level

Data are presented as mean ± SEM, n=6. Control, Negative control (Acetic acid induced), Positive Control (Prednisolone), Low Dose, High Dose of ELECM. One-way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * p<0.05, ** p<0.01, *** p<0.001

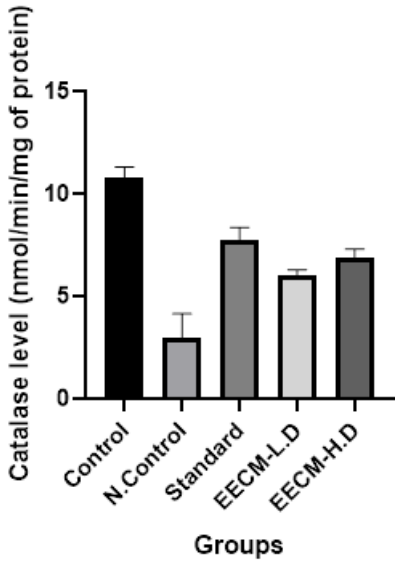


**Fig.-4:** Effect of ELECM on colon tissue catalase level

Data are presented as mean ± SEM, n=6. Control, Negative control (Acetic acid induced), Positive Control (Prednisolone), Low Dose, High Dose of ELECM. One-way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * p<0.05, ** p<0.01, *** p<0.001

acetic acid-treated rats with 140.72 ± 9.00, reflecting close to 50% reduction of all treatment group as compared to control (Table 3 and Fig. 5).

The anti-inflammatory effect was reinforced further by a substantial reduction of colon MDA levels. The tissue MDA of standard, low dose of test, and high dose of the test were 22.15 ± 0.49, 19.59 ± 0.29, and 17.69 ± 0.26 (nM/mg of protein), respectively (Table 3 and Fig. 6), as compared to elevated level on the acetic-acid induced group as 28.87 ± 0.61 (nM/mg of protein).

**Table-3:** Effect of ELECM on colon lipid peroxidase inhibitory effect

| *S. No* | *Groups* | *Colon MPO activity (µg/mg of tissue)* | *Colon MDA activity (nM/mg of protein)* |
|---|---|---|---|
| 1 | Control | 10.32 ±0.23 | 11.32 ±0.48 |
| 2 | Negative | 140.70 ± 9.00 | 28.87 ± 0.61 |
| 3 | Positive | 80.53 ± 2.96 | 22.15 ± 0.49 |
| 4 | LD ELECM | 76.33 ± 2.66 | 19.59 ± 0.29 |
| 5 | HD ELECM | 62.76 ± 2.41 | 17.69 ± 0.26 |

Data are presented as mean ± SEM, n=6. Control, Negative control (Acetic acid induced), Positive Control (Prednisolone), Low Dose, High Dose of ELECM. One-way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * p<0.05, ** p<0.01, *** p<0.001

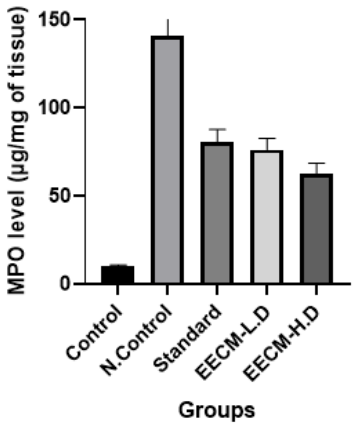


**Fig. 5:** Effect of ELECM on colon tissue MPO level

Data are presented as mean ± SEM, n=6. Control, Negative control (Acetic acid induced), Positive Control (Prednisolone), Low Dose, High Dose of ELECM. One-way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * p<0.05, ** p<0.01, *** p<0.001

## Immune Markers (Serum TNF-α and IL-6) Levels

The TNF-α concentration of the disease control group was found to be 73.60 ± 1.04 pg/mL as compared to the control group of 16.44 ± 0.28 pg/mL, implying about 77.72% increased level of TNF-α (Table 3 and Fig. 7). However, the standard and low dose and high dose of the test were found to be 44.09 ± 1.28, 61.02 ± 0.80 and 55.43 ± 0.63 pg/mL, respectively and the %increase of about 62.81, 73.13 and 70.42. The results indicate a very mild reduction in the level of TNF-α almost in all treatment groups.

The concentration of IL-6 in disease control, standard, low dose, and high dose test was 31.24 ± 0.56, 15.45 ± 0.2031, 21.45 ± 0.85, and 18.54 ± 0.39 pg/mL, respectively, and their % increase level of IL-1 was 72.31, 44.08, 59.72 and 53.34, respectively (Table 3 and Fig. 8), indicates a low reduction of IL-6 almost in all treatment group.

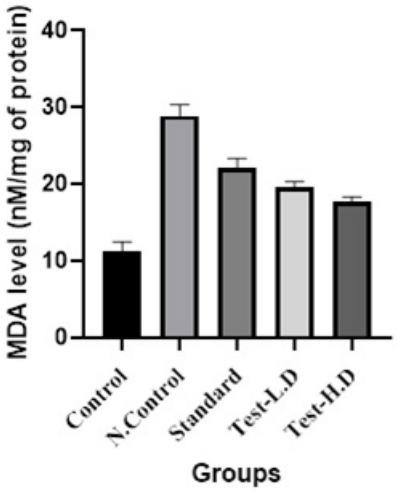


**Fig. 6:** Effect of ELECM on colon tissue MDA level

Data are presented as mean ± SEM, n=6. Control, Negative control (Acetic acid induced), Positive Control (Prednisolone), Low Dose, High Dose of ELECM. One-way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * p<0.05, ** p<0.01, *** p<0.001

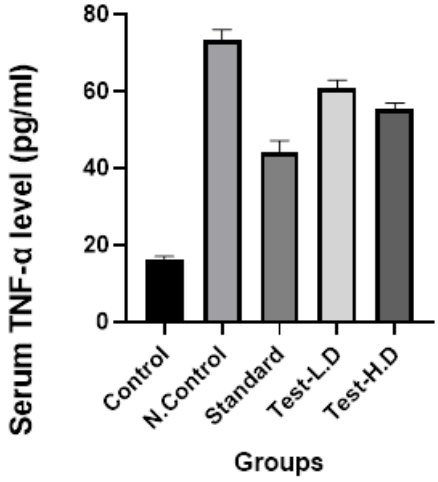


**Fig. 7:** Effect of ELECM on serum TNF-α level

Data are presented as mean ± SEM, n=6. Control, Negative control (Acetic acid induced), Positive Control (Prednisolone), Low Dose, High Dose of ELECM. One-way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * p<0.05, ** p<0.01, *** p<0.001

### Microscopical Examination of Colon and Caecum

The microscopical examination of the colon and caecum of the acetic-acid-induced ulcerative colitis group showed ulcerative mucosa and dense lymphatic infiltration however the standard and test treatment group illustrate there was only mild ulcer and lymphatic infiltration, implying the test group has effective in reducing the symptoms of ulcerative colitis similar as standard drug prednisolone.

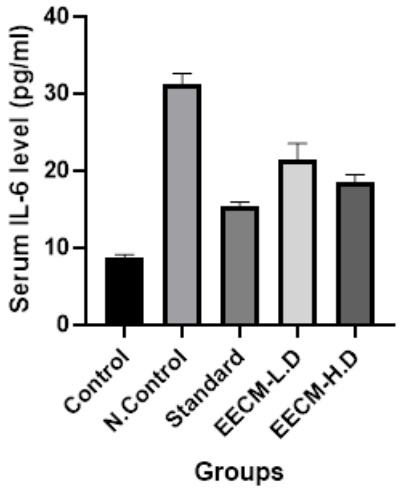


**Fig. 8:** Effect of ELECM on Serum IL-6 level (pg/mL)

Data are presented as mean ± SEM, n=6. Control, Negative control (Acetic acid induced), Positive Control (Prednisolone), Low Dose, High Dose of ELECM. One-way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * p<0.05, ** p<0.01, *** p<0.001

## Discussion

The present study aimed to assess the efficacy of ELECM to treat acetic acid-induced enterocolitis in wistar rats, though it is an acute study the pathological marker and the signs and symptoms imitate the human being. A higher and almost the same extent of antioxidant activity was observed through decreased levels of SOD and CAT in rat colon on both standard (prednisolone) and test groups (ELECM) compared to acetic acid-induced ulcerative colitis treated (negative group) rats.

The lipid peroxidation, and was significantly increased in the negative group rats as compared with the group treated with prednisolone (standard) group and ELECM treated (test group) of acetic acid-induced ulcerative colitis animal model and also pro-inflammatory mediators such as IFN-α and IL-6 indicate there was about mild to moderate immuno-suppressive response as compared to the standard drug treatment prednisolone. These findings illustrate, that there is a potent and moderate anti-inflammatory response for the standard and ELECM test groups, respectively similar results also reflected by the reduction of colon weight of the test group as compared to the diseased control.

The histological examination of the acetic-acid group shows ulcerative mucosa and the lamina propria showed dense lymphocytic infiltration with cryptitis and crypt abscess. However the ELECM-treated test group showed nearly normal epithelium, mild infiltrate, and mild cryptitis similar to the prednisolone-treated (standard group), indicating there is no significant pathology for the ELECM-treated test group. The GC-MS analysis of leaves ELECM confirms the presence of about 12 specific phytochemicals

**Table 4:** Effect of ELECM on colon immune-suppresive effect

| S. No | Groups | Serum TNF-α level (pg/mL) | Serum IL-6 level (pg/mL) |
|---|---|---|---|
| 1 | Control | 16.4 ± 0.28 | 8.65 ± 0.19 |
| 2 | Negative | 73.6 ± 1.04 | 31.24 ± 0.57 |
| 3 | Positive | 44.09 ± 1.29 | 15.45 ± 0.20 |
| 4 | LD ELECM | 61.02 ± 0.80 | 21.45 ± 0.85 |
| 5 | HD ELECM | 55.43 ± 0.63 | 18.54 ± 0.39 |

Data are presented as mean ± SEM, n=6. Control, Negative control (Acetic acid induced), Positive Control (Prednisolone), Low Dose, High Dose of ELECM. One-way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * p<0.05, ** p<0.01, *** p<0.001

**Table 5:** ELECM GC-MS photochemical and reported pharmacological activities

| *S. no* | *Photochemicals* | *Pharmacological activity* |
|---|---|---|
| 1 | 5-Hydroxymethylfurfural | Antioxidant and Anti-cancer (32) |
| 2 | 2-Methoxy-4-vinylphenol | Antioxidant and antimicrobial (33) |
| 3 | 1H-Inden-1-one, 2,3-dihydrotetramethyl- | Analgesic and CNS Stimulant and depressant activity (34) |
| 4 | Bergamotol, Z-.alpha.-trans- | Antioxidant and Anti-microbial activity (35) |
| 5 | Tetradecanoic acid | Anti-microbial (36) |
| 6 | Neophyadiene | Anti-inflammatory (37) |
| 7 | Hexadecanoic acid | Antioxidant and anti-inflammatory activity (38) |
| 8 | Ethyl palmitate | Anti-inflammatory activity (39) |
| 9 | 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol | Antioxidant and Hypo-lipdemic activity (40) |
| 10 | Alpha-Linolenic acid | Antioxidant and anti-inflammatory and anticancer activity (41) |
| 11 | 9-Octadecenoic acid (Z)- | Motor activity (42) |

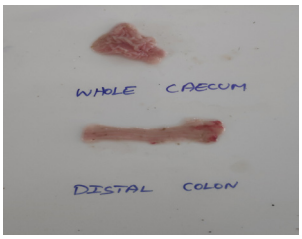


**Fig. 9A:** (Control Group)

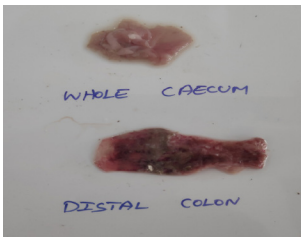


**Fig. 9B:** (Negative Group)

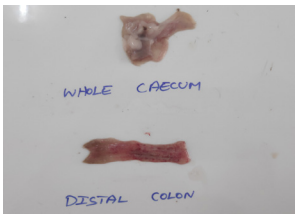


**Fig. 9C:** (Standard Group)

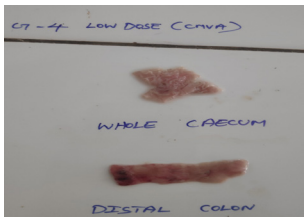


**Fig. 9D:** (Test-Low Dose)

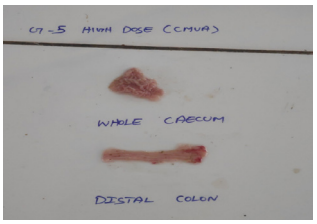


**Fig. 9E:** (Test - High Dose)

**Fig. 9:** Images of caecum and colon in acetic acid induced enterocolitis

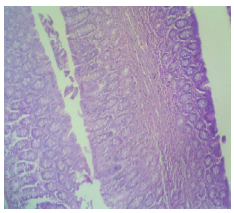

**Fig. 10A:** (Control) Colon shows normal mucosa. Lamina propria shows scattered lymphocytic infiltrates. Serosa and muscular layer show scattered inflammatory infiltrates. There is no granuloma/ malignancy seen

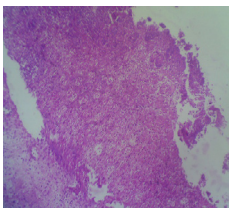

**Fig. 10B:** (Negative Control) Colon shows ulcerative mucosa. The lamina propria shows dense lymphocytic infiltration with cryptitis and crypt abscess. Muscularis layer and serosa shows inflammatory infiltrates

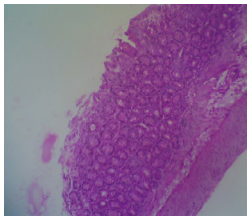

**Fig. 10C:** (Standard) Colon shows normal epithelium one focal shows ulcerated epithelium. The lamina propria is edema and inflammatory infiltrates composed of lymphocytes and neutrophils and focal cryptitis is also seen. Sub mucosa is mild edema. Muscularis layer shows no significant pathology

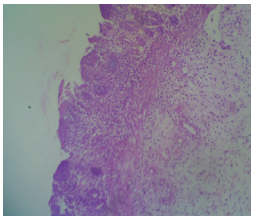

**Fig. 10D:** (Test - Low Dose) Colon shows normal epithelium one focal shows ulcerated epithelium. The lamina propria is edema and inflammatory infiltrates composed of lymphocytes and neutrophils and focal cryptitis is also seen. Sub mucosa is mild edema. Muscularis layer shows no significant pathology

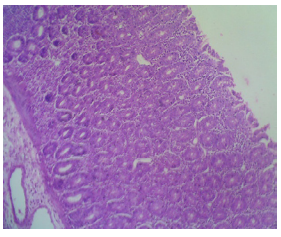

**Fig. 10E:** (Test - High Dose) Colon shows normal epithelium one focal shows ulcerated epithelium. The lamina propria is edema and inflammatory infiltrates composed of lymphocytes and neutrophils and focal cryptitis is also seen. Sub mucosa is mild edema. Muscularis layer shows no significant pathology

**Fig.-10:** Acetic acid - histopathology of colon

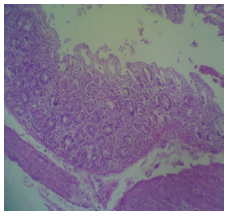

**Fig. 11A:** (Control)

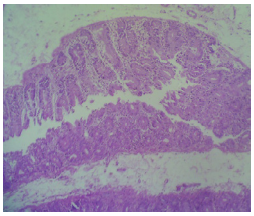

**Fig. 11B:** (Negative Control)

Caecum shows ulcerated epithelium. Lamina propria shows lymphocytic infiltrates areas shows cryptitis and dense inflammatory infiltrates seen. Muscular layer and serosa show dense inflammatory infiltrates. Mucosal layer shows focal fibrosis.

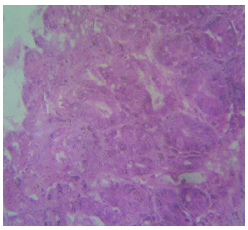

**Fig. 11C:** (Standard)

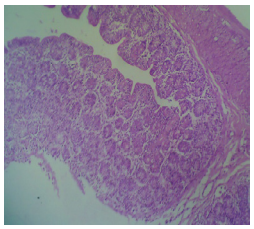

**Fig. 11D:** (Test - Low Dose)

Caecum shows normal epithelium. Lamina propria shows few scattered lymphocytic infiltrates and connective tissues. There is no malignancy/granuloma seen.

Caecum shows normal epithelium. Lamina propria shows few scattered lymphocytic infiltrates and connective tissues. There is no malignancy/granuloma seen.

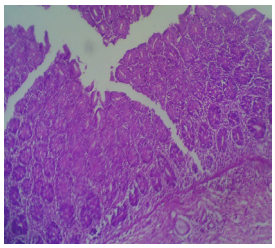

**Fig.-11E:** (Test - High Dose)

Caecum shows normal epithelium. Lamina propria shows few scattered lymphocytic infiltrates and connective tissues. There is no malignancy/granuloma seen.

**Fig. 11:** Acetic acid - histopathology of caecum

(Fig. 1), among the compounds represented[35-45] in Table 5. The compound number 1, 2, 4, 7, 9 and10 has antioxidant effect and the compound number 7, 8 and 10 possessing anti-inflammatory activity might be responsible for the anti-ulcerative activity.

## Conclusion

All the parameters of ELECM-treated test group animals had shown better results which are comparable with the standard prednisolone-treated group. Therefore, our present study suggests that ELECM extract has significant protective activity against acetic acid-induced ulcerative colitis, due to its higher antioxidant, moderate anti-inflammatory, and mild immuno-suppressive effects. The protective action of this ELECM may be due to the presence of active constituents such as flavonoids and total phenols as presented on GC-MS analysis.

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