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Research Article

Effect Of Clerodendrum Serratum L. Extract In Chemically Induced Ulcerave Colis In Rats

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ARTICLEINO

ABSTRACT

In this study, we invesgated the impact of *Clerodendrum serratum* L. on chemically induced ulcerave colis in rats. The roots of *Clerodendrum serratum* L. were ulized to extract crude methanol for in vivo acvity studies. Ulcerave colis was induced in rats using 25mg TNBS in 50% ethanol via the intrarectal route. Animals were divided into various treatment groups, including a normal control group, a disease control group treated with normal saline, and groups treated with different doses of *Clerodendrum serratum* L. extract (100mg, 200mg, 400mg) for seven days. Sulfasalazine (120mg/kg) served as the standard drug. Daily monitoring of parameters such as body weight, food-water intake, rectal bleeding, and diarrhoea was conducted. Aer the treatment period, animals were sacrificed for evaluaon of colon length-weight, CMDI, histopathology, and anoxidant parameters (superoxide dismutase, catalase, nitric oxide, and lipid peroxidase acvity). Results indicated a significant improvement in various parameters, suggesng the potenal therapeuc efficacy of *Clerodendrum serratum* L. extract in TNBS-induced ulcerave colis in rats.

Keywords: *Ulcerative colitis, TNBS, Methanolic extract of Clerodendrum serratum L., Anti- oxidant activity, Inflammation.*

1 Introducon

Inflammatory bowel disease (IBD) encompasses chronic idiopathic inflammatory condions affecng the gastrointesnal tract, notably ulcerave colis (UC) and Crohn's disease (CD). UC manifests as connuous inflammaon localized to the colon and rectum, involving only the mucosal layer. Conversely, CD exhibits inflammaon in various parts of the gastrointesnal tract, displaying disconnuous or "patchy" lesions that affect all layers of the intesnal wall. The eology of ulcerave colis remains elusive, but genec and environmental factors are implicated. Dysregulaon of the intesnal immune system, coupled with altered microorganisms and increased intesnal permeability, contributes to gastrointesnal damage. UC prevails more in India compared to CD. Countries that have adopted an industrialized lifestyle in that incidence risk has increased, which suggests that environmental factors might be crucial in the triggering of disease onset.[5] Common symptoms associated with ulcerave colis include diarrhoea, abdominal pain, bloody stools, weight loss, and fague [5]. The chronic nature of UC leads to a relapsing and reming patern, significantly impacng the quality of life for affected individuals.

Convenonal treatments for UC include mesalazine(5-ASA), corcosteroids, immunosuppressive agents, and TNF-α monoclonal anbodies. Despite their efficacy, these medicaons are costly and associated with long-term side effects, liming their ulity. Herbal medicines, with their potenal benefits and minimal side effects, emerge as cost-effecve alternaves. *Clerodendrum serratum* L., a tradionally esteemed plant in India, has a history of applicaon in treang various ailments, including pain, inflammaon, rheumasm, respiratory disorders, and fever. *Clerodendrum serratum* L. contains saponins (terpenoids and steroids), flavonoids and phenolics and carbohydrate. Roots of *Clerodendrum serratum* L. are reach source of oleanolic acid, ursolic acid, β – sitosterol.[18] Ursolic acid has been reported for having an - inflammatory acvity by inhibing the NF- kB and decrease the level of TNF-α. [38,39] Oleanolic acid is also reported for having an-inflammatory acvity, it inhibits the pro – inflammatory effect by down regulang NF- kB and TNF-α. [39] β – sitosterol has been reported for having an- inflammatory acvity. So that, its ulizaon presents a promising avenue for the development of effecve, affordable, and well-tolerated treatments for ulcerave colis.

2 Materials and methods:

Plant collection & Authentication
Dry *clerodendrum serratum* L. roots was purchased from JK Botanicals Pvt Ltd, Mumbai. Authencaon of plants was done at Department of Biosciences, Saurashtra University, Rajkot.

Plant Extraction
Dried roots were coarsely crush and powdered. Powder was extracted using 80% methanol at 60 ℃ in Soxhlet apparatus for 16 hrs. and concentrated by evaporaon process. Obtained crude extract was filtered by Whatman no. 1 filter paper into a conical flask and allowed to dry at room temperature. Crude extract was stored in closed container at 4 ℃

Experimental Animals
Experiment was conducted according to CCSEA (CPCSEA)guideline and the study was approved by Instuonal animal ethics commitee (protocol no.
BKMGPC/ISEC29/RP100/2022). Either sex Animals (Rats) was used and maintained under standardized condion and provide pelleted diet and purified drinking water. They were housed at ambient temperature (22±1℃), relave humidity (55±5%) and 12h/12h light dark cycle. Throughout the experiments, animals were process according to the suggested ethical guideline for the care of laboratory animals.

Induction & Treatment of Colitis
Animals was divided in six groups, 6 animals in each group. On first day, TNBS was administered intra-rectally to all the groups except normal control group for inducon of ulcerave colis. Methanolic extract of *Clerodendrum serratum* L. roots was administered through oral route for consecuve seven days in treatment groups. Normal control group and disease control group was received vehicle through oral route. Change in body weight was monitored for 8 days. On day 8, animals were sacrificed and evaluaon parameters was performed.

Clinical Assessment of Colitis

Daily measurement of Body weight
Clinical measures of colis induced by TNBS included daily determinaon of body weight. The difference between the inial and final weight was used to determine body weight loss. Body weight loss suggests the onset of ulcerave colis.

Measurement of Colon Length and Weight [48]
On day 8, animals were sacrificed. To isolate the colon, each rat was dissected. The proximal rectum was separate from the colons near its passage under the pelvisternum. Colon was washed with ice-cold phosphate buffer saline immediately (pH 7.4). Colon length was determined by laying it on graph paper with a standard-length measurement in cenmetres. To calculate severity, the weight of colon ssue was measured and compared.

Colon mucosal damage index [36,49]
Distal 10 cm of colon segment was exercised, opened by midline incision and rinsed with the saline. It was observed from luminal side. Microscopic scoring was done as shown in table 1.

Table 1 Score of Colon mucosal damage index

Score	Damage
0	Normal mucosa, no damage on mucosal surface
1	Mild Hyperemia and edema, no erosion or ulcer on mucosal surface
2	Moderate Hyperemia and edema
3	Severe Hyperemia and edema with major ulcerative area <1 cm
4	Severe Hyperemia and edema, necrosis and ulcer appearing on the mucosal surface with major ulcerative area >1 cm

Disease Activity Index [48,50]
The DAI score was calculated using three major clinical symptoms: weight loss, diarrhoea and rectal bleeding.
DAI = Weight loss + rectal bleeding score + diarrhoea score

Weight loss:
Percentage difference between an actual and expected body weight on a parcular day was considered as weight loss.

A 0-4 score was given using a formula menoned below:
Diarrhoea:
It can be defined as mucus /faecal material sck to anal fur. Range of 0- 4 score will be assigned according to presences of diarrhoea as shown in table 5.2 Rectal bleeding:
Diarrhoea consisng of mucus or blood can be defined as a rectal bleeding. Range of 0-4 score was assigned according to presence of rectal bleeding as shown in table 2.

Score	Weight loss	Stool Consistency	Bleeding
0	Normal	Normal (Well- formed pellets)	Not observed
1	1-5%	Normal	Not observed
2	6-10%	Loose (Pasty stools that don't stick to anus)	Occult
3	11-15%	Loose (Pasty stools that don't stick to anus)	Occult
4	>15%	Diarrhoea (Liquid stools that don't stick to anus)	Gross bleeding

Table 2 Score of weight loss, stool consistency and bleeding

Evaluation of Antioxidant Parameters
Extracted colon ssue was homogenized in ice-cold tris hydrochloride buffer (10mM, pH 7.4). The homogenate was centrifuged for 20 minutes at 7000 rpm in a homogenizer, and the supernatant was analysed to calculate oxidave stress parameters.

Total Protein Esmaon [51]
1ml colon ssue homogenate was added to several test tubes, then diluted with 0.1N NaOH to 1.5 ml.1.5 ml alkaline copper soluon was added to this soluon, mixed thoroughly, and set aside for 10 minutes. With constant shaking, 0.5 ml diluted Follin's reagent was added to the aforesaid soluon. Allow 30 minutes for the test tubes to stand. Colour was generated and absorbance was measure at 750nm in UV Spectrophotometer. Total protein was measure in milligrams per gramme of ssue.

Superoxide Dismutase (SOD)
0.2 ml 10 percent w/v ssue homogenate was added to2.5 ml of 0.05M carbonate buffer (pH 10.2). The reacon was started by adding 0.5 mL epinephrine to this supernatant. The opcal density of synthesized adenochrome was monitored at 480 nm every 30 seconds for 3 minutes. Under specific test condions, one unit of enzyme acvity was defined as the concentraon required to suppress chrome synthesis by 50% in one minute. Results of Enzyme acvity are expressed in UI/mg protein.

Catalase level [53]
This catalase assay is based on the ability of catalase to induce the disappearance of H2O2. Using phosphate buffer, homogenate was diluted 20 mes. 1 ml phosphate buffer was added to 1 ml diluted homogenate to make the test soluon. Just before measuring opcal density, a 1 ml H2O2 soluon was injected. Absorbance was measured at 240 nm for 3 minutes at 15 second intervals. Catalase acvity was determined using the molar exncon coefficient of H2O2 of 43.6M-1 cm1. 1m/mol H2O2 degraded every minute equals one unit of acvity. CAT acvity was expressed as a UI/mg protein.

Nitric Oxide (NO) Acvity [47]
The Griess reacon was used to determine the amount of accumulated nitrite in the homogenate. Nitrite and nitrate producon is an indicator of NO synthesis, was measured by using Griess reagent. At room temperature, 100 µl samples was incubated for 10 minutes with 100 µl Griess reagent. Absorbance was measured at the 540nm. The standard curve was developed using a known concentraon of sodium nitrite. The amount of nitrate release was quanfied by comparison with standard sodium nitrite.

Lipid Peroxidaon (LPO) Acvity [50]
Lipid peroxidase was evaluated by measuring malondialdehyde (MDA) level.

0.5 ml colon ssue homogenate was added to 0.5ml of 20% TCA and 1ml of 0.67% TBA. Then was boiled at 100 °C for 10 min, cooled immediately in ice bath and added 4ml n- butanol. Then centrifuged at 3000 rpm for 15min, supernatant was collected and absorbance was measured at 530 nm. The amount of MDA was expressed as nmol/g of colonic ssue protein.

Histopathology [50,54,55]
Colon ssue samples was kept in 10% formalin, then embedded in paraffin at a thickness of 5µm and stain with haematoxylins and eosin. A microscope was used to perform histopathological evaluaon parameters.

Statistical Analysis
All values were expressed as mean ± SEM. The stascal significance was tested by one way & two-way analysis of variance (ANOVA) followed by Tukey test used for colon mucosal damage index (CMDI). $P < 0.05$ was considered as significant difference among the two different groups.

3 Results

Qualitative phytochemical analysis
The percentage yield of methanolic extract of 60 gm of dried powder of roots of *Clerodendrum serratum* L. was found to be 9.96% w/w using Soxhlet method of extracon. The idenficaon of major secondary metabolites of methanolic extract was carried out to determine the presence or absence of the different phytoconstuents. The results were evaluated by visual inspecon as a change in color or precipitaon. Table 3 shows the presence or absence of phytoconstuents in plant extract.

Table 3 Phytochemical analysis of methanolic extract of plant

Phytoconstituents	Result
Alkaloids	-
Flavonoids	+
Carbohydrates	+
Protein and amino acid	-
Tannins	-
Glycosides	-
Steroids and triterpenoids	+
Phenols	+

Change in Body Weight
From the 3rd day onwards a body weight was observed to be significantly ($P<0.05$) lower in of animals of disease control group as compared to normal control group. Treatment with MECS (200 & 400mg/kg) shown significantly higher body weight of animals as shown in figure 1. These both group of animals were shown to have significant ($P<0.05$) lower reducon in body weight as compared to disease control group. Standard drug (Sulfasalazine, 120 mg/kg)

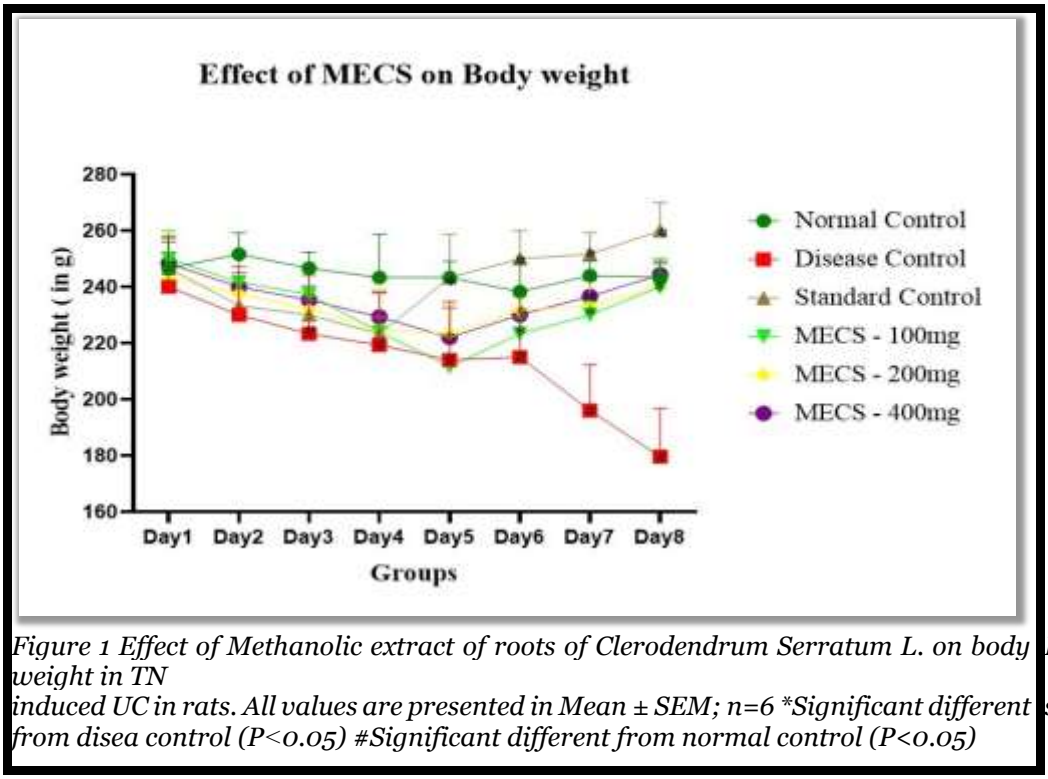


Figure 1 Effect of Methanolic extract of roots of Clerodendrum Serratum L. on body BS weight in TN
*induced UC in rats. All values are presented in Mean ± SEM; n=6 *Significant different se from disea control (P<0.05) #Significant different from normal control (P<0.05)*

also shown significantly improved (P<0.05) body weight as compared to disease control group as shown in figure 1.

Change in water intake

A significant reducon (P<0.05) in water intake was observed from the 3rd day onwards in disease control group as compared to normal control group. Standard drug (Sulphasalazine, 120 mg/kg) treated animals shown significantly higher (P<0.05) water intake as compared to disease control group. Treatment with MECS (200 & 400 mg/kg) shown higher water intake in animals of both groups as compared to disease control group (P<0.05) as shown in figure 2.

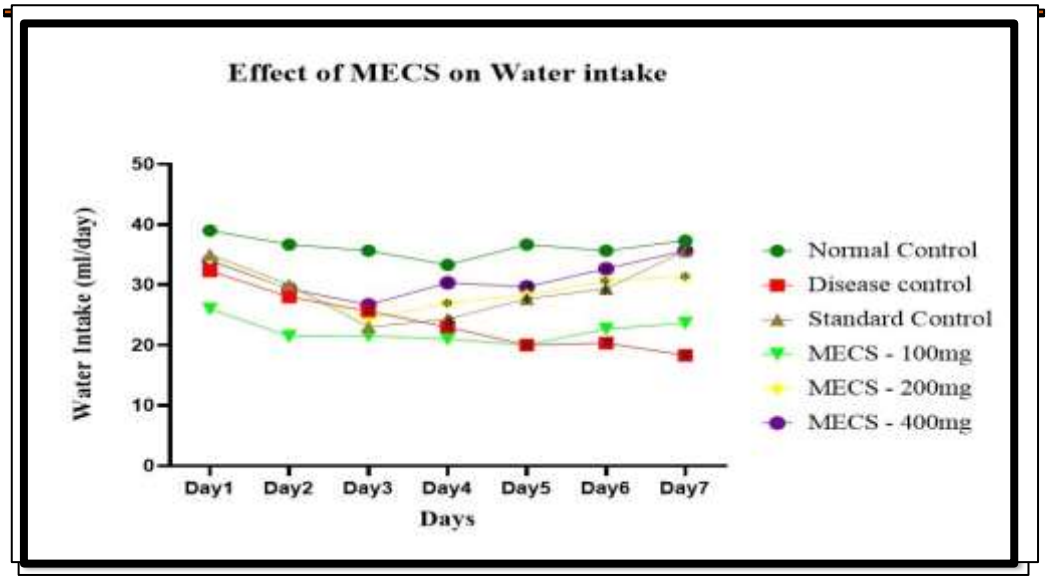


*Figure 2 Effect of Methanolic extract of roots of Clerodendrum serratum L. on water intake in TNBS induced UC in rats. All values are represented in Mean ± SEM; n=6 *Significant different from disease control (P<0.05) #Significant different from normal control (P<0.05)*

Change in Food Intake

A significant reducon (P<0.05) in food intake was observed in disease control group as compared to normal control group. Administraon of TNBS lowered food intake as compared to normal control group. Standard drug (Sulphasalazine, 120 mg/kg) treated animals shown significantly higher food intake as compared to disease control group. Treatment with MECS (200 & 400 mg/kg) shown significantly higher food intake as shown in figure 3. These both group of animals were shown to have significant higher (P<0.05) food consumpon as compared to disease control group.

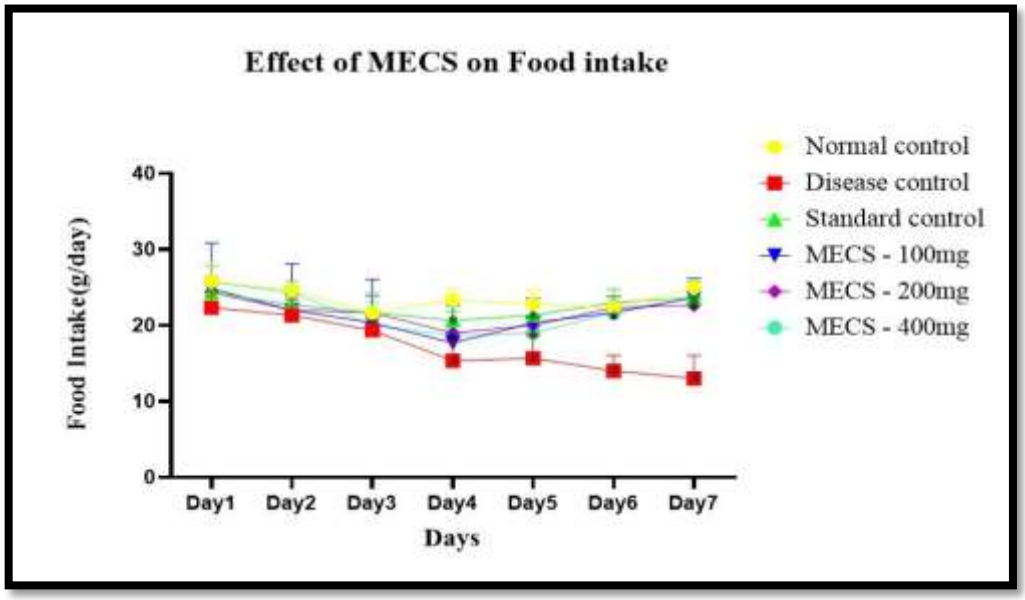
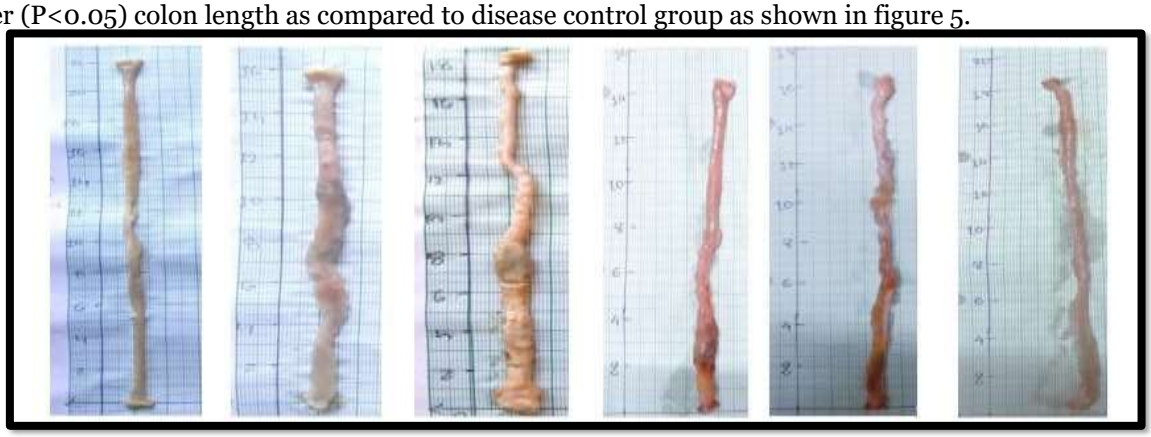


*Figure 3 Effect of Methanolic extract of roots of Clerodendrum serratum L. on food intake in TNBS induced UC in rats. All values are represented in Mean ± SEM; n=6 *Significant different from disease control (P<0.05) #Significant different from normal control (P<0.05)*

Colon Length

A Significant lower (P<0.05) colon length was observed in animals of disease control group as compared to normal control group. Treatment with MECS (200 & 400 mg/kg) shown higher colon length as shown in figure 4. These both group of animals were shown to have significant (P<0.05) higher colon length as compared to disease control group. Standard drug (Sulphasalazine, 120 mg/kg) treated animals also shown significantly higher (P<0.05) colon length as compared to disease control group as shown in figure 5.



NC DC SC MECS(100mg) MECS(200mg) MECS

Figure 4 Effect of MECS on colon length in TNBS induced UC in rats (400mg)

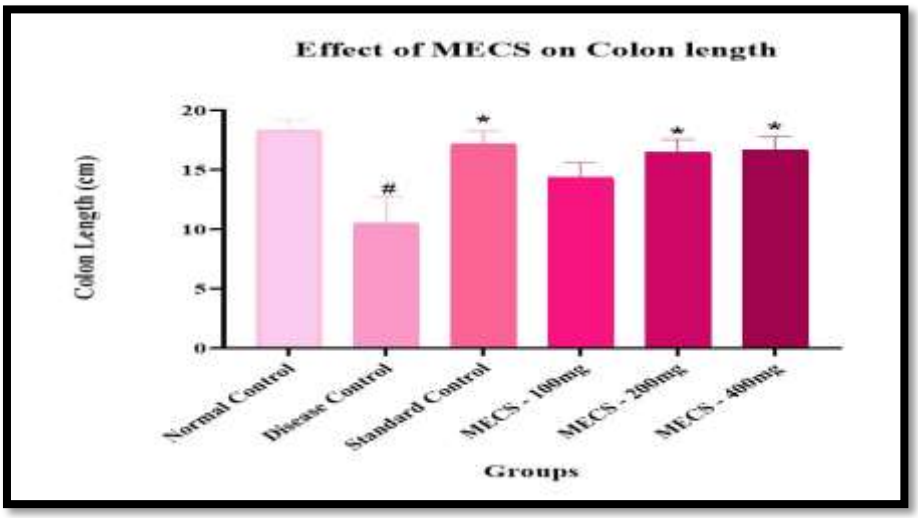


*Figure 5 Effect of Methanolic extract of roots of Clerodendrum serratum L. on colon length in TNBS induced UC in rats. All values are represented in Mean ± SEM; n=6 *Significant different from disease control (P<0.05)*
#Significant different from normal control (P<0.05)

Colon Weight
The disease control group had a significantly lower weight (P<0.05) than the normal control group. MECS (400mg/kg) treatment resulted in higher colon weight, as shown in the table 6. When compared to animals of disease control group, these groups of animals had a significantly higher(P<0.05) colon weight. As shown in the figure 6 the standard drug (Sulphasalazine, 120 mg/kg) resulted in much higher weight than the disease control group.

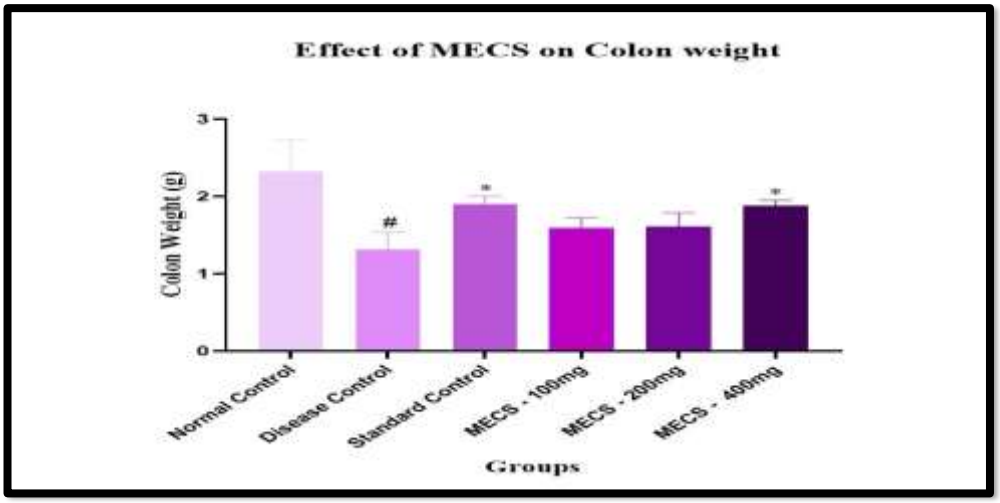


*Figure 6 Effect of Methanolic extract of roots of Clerodendrum serratum L. on colon weight in TNBS induced UC in rats. All values are represented in Mean ± SEM; n=6 *Significant different from disease control (P<0.05) #Significant different from normal control (P<0.05)*

Colon Mucosal Damage Index
The colon ssue damage score in disease control group had a significantly higher (P<0.05) CMDI score than the normal control group. The animals' CMDI scores were lower aer treatment with MECS (400 mg/kg), as shown in the figure7. MECS (400mg/kg) treated group showed significant reducon in CMDI score as compared to disease control group (P<0.05).

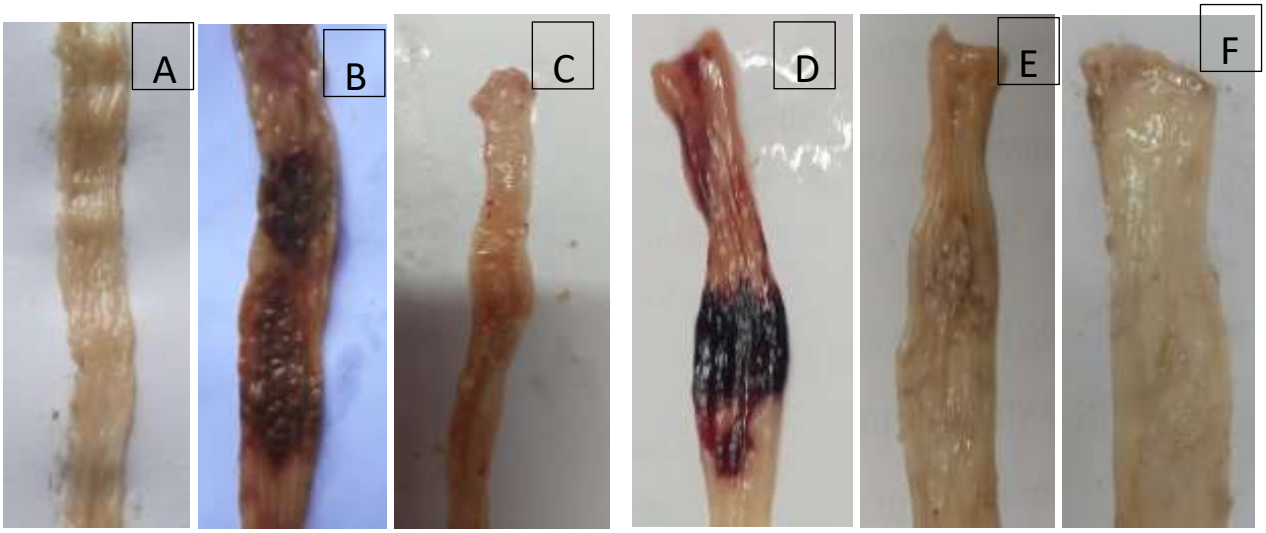


Figure 8 Macroscopic presentation of TNBS – induced ulcerative colitis. (A) Normal colon treated with normal saline, (B) TNBS – treated colon with 25mg/kg, (C) colon treated with standard sulfasalazine dose120mg/kg, (D) colon treated with 100mg/kg MECS, (E) colon treated with 200mg/kg MECS, (F) colon treated with 400mg/kg MECS.

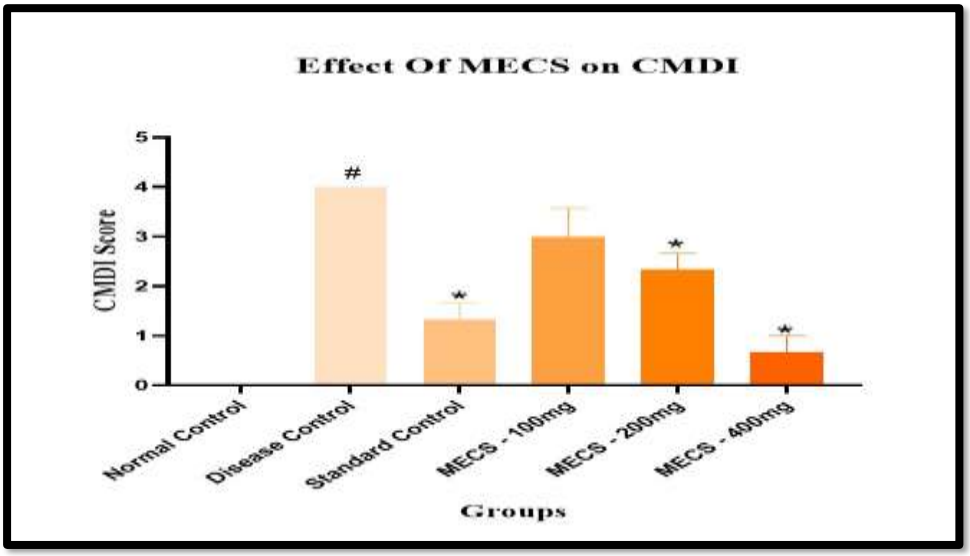


*Figure 7 Effect of Methanolic extract of roots of Clerodendrum serratum L. on CMDI in TNBS induced UC in rats. All values are represented in Mean ± SEM; n=6 *Significant different from disease control (P<0.05) #Significant different from normal control (P<0.05)*

Disease Activity Index

From the fourth day onwards, the animals of disease control group showed more severe symptoms and had a significantly higher (P<0.05) DAI score than the normal control group.In case of MECS (400 mg/kg) treated group results showed a significant (P<0.05) lower DAI score than those in the disease control group.

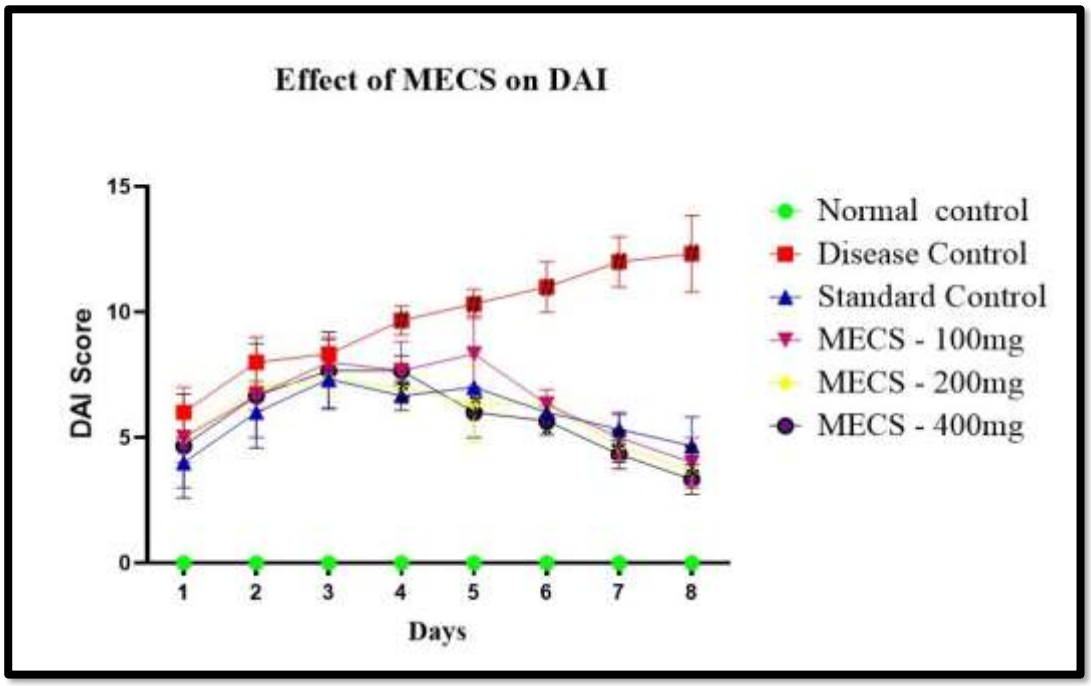
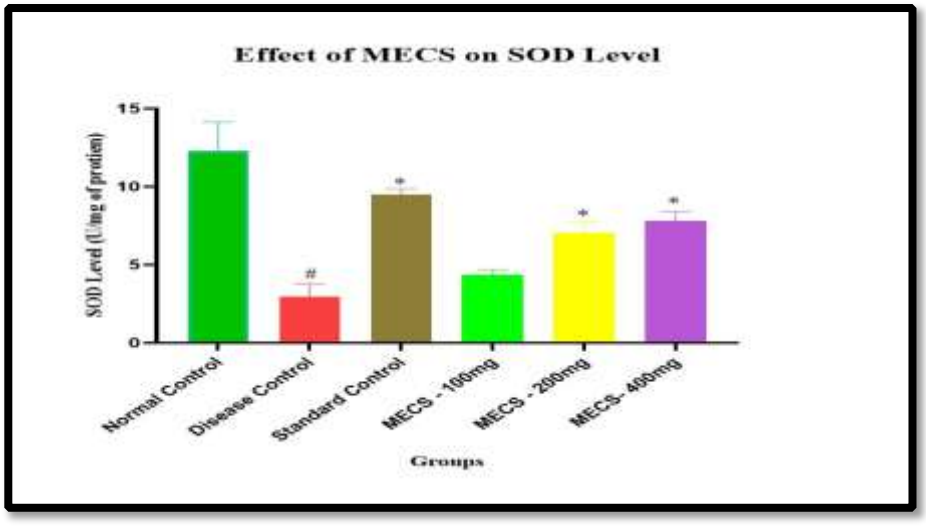


*Figure 9 Effect of Methanolic extract of roots of Clerodendrum serratum L. disease activity index in TNBS induced UC in rats. All values are represented in Mean ± SEM; n=6 *Significant different from disease control (P<0.05) #Significant different from normal control (P<0.05)*

Standard drug (Sulphasalazine, 120 mg/kg) shown significantly lower (P<0.05*) DAI score as compared to disease control group as shown in figure 9.*

Superoxide dismutase (SOD) level

Superoxide dismutase (SOD) level was significantly (P<0.05) lower in colons of TNBS treated experimental animals as compared to normal control (NC) group as shown in figure 10. Standard drug (Sulphasalazine, 120 mg/kg) and MECS (400 mg/kg) treated group shown significant higher (P<0.05) level of SOD as compared to disease control (DC) group.



Catalase level

In the present study the catalase level was assessed in all the experimental animals is showed in the fig.11. results reveal that animals of disease control *Figure 10 Effect of Methanolic extract of roots of Clerodendrum serratum L. on SOD level in* groups shows the significant (P<0.05) lower level of catalase as compared to *TNBS induced UC rats. All values are represented in Mean ± SEM; n=6 *Significant different* normal control (NC) group. Treatment with Methanolic extract of roots of *from disease control (P<0.05) #Significant different from normal control (P<0.05)*

Clerodendrum serratum L.(100mg/kg) shown no change level of catalase as compared to disease control group as shown in figure 11. Treatment with methanolic extract of roots of Clerodendrum serratum L. (200mg/kg

and 400 mg/kg) resulted in significantly higher (p<0.05) catalase level. Animals of both groups were shown to have significant higher (P<0.05) level of catalase as compared to disease control group.

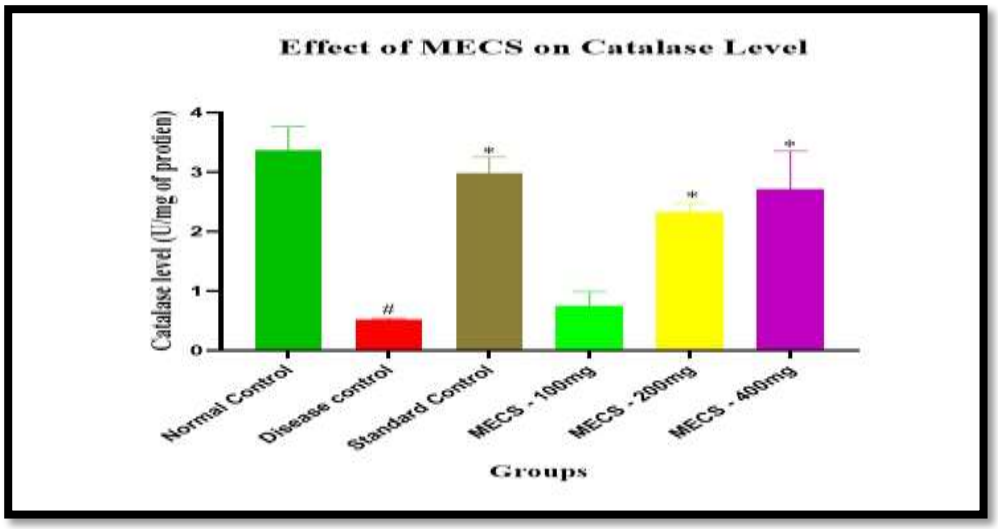


*Figure 11 Effect of Methanolic extract of roots of Clerodendrum serratum L. on catalase level in TNBS induced UC rats. All values are represented in Mean ± SEM; n=6. *Significant different from disease control (P<0.05) #Significant different from normal control (P<0.05)*Significant different from disease control (P<0.05) #Significant different from normal control (P<0.05)*

Nitric oxide (NO) Activity

Aer TNBS treatment nitric oxide producon significant higher (P<0.05) in disease control (DC) group compared to normal control group (NC) as shown in figure 12. Standard drug (Sulphasalazine, 120 mg/kg) shown significant lower(P<0.05) level of nitric oxide as compared to disease control group as shown in figure 12. However, MECS treatment at dose level (200 & 400 mg/kg) resulted in significantly lower nitric oxide (NO) as compared to animals of disease control group.

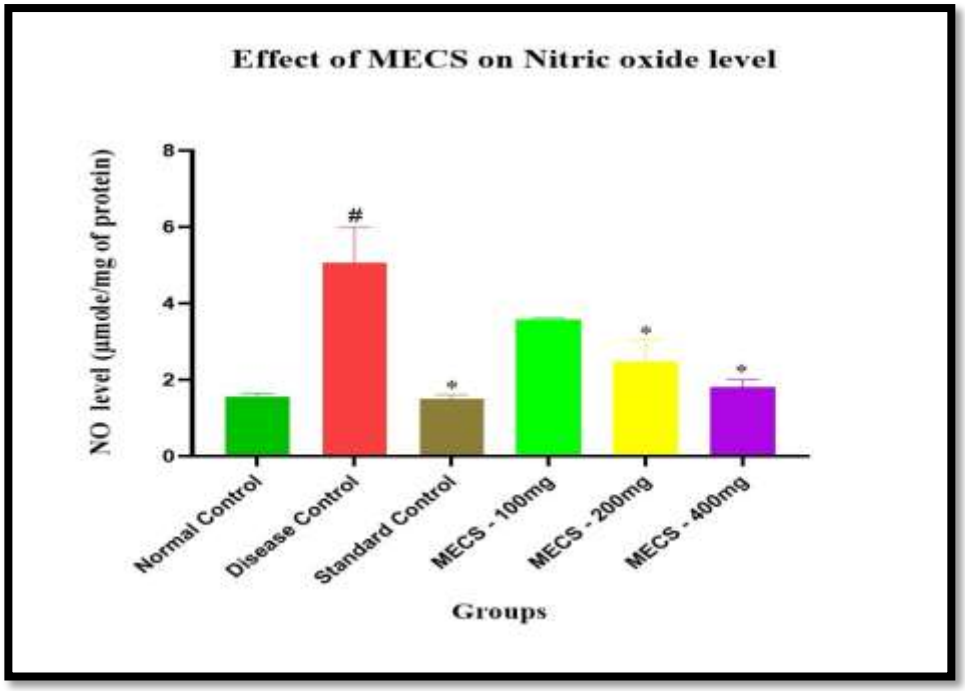


*Figure 12 Effect of Methanolic extract of roots of Clerodendrum serratum L. on nitric oxide level in TNBS induced UC rats. All values are represented in Mean ± SEM; n=6. *Significant different from disease control (P<0.05) #Significant different from normal control (P<0.05)*

Lipid peroxidation (LPO) Activity

Lipid peroxidaon, an indicator of mucosal injury induced by the ROS. The MDA is the product of lipid peroxidaon. In this study change in MDA (malondialdehyde) content normal control group and MECS treated animals showed in fig no. 13. A Significant higher (P<0.05) MDA level was observed in disease control group

as compared to normal control group which indicates neutrophil infiltraon in colon. No change was observed in MDA level in between disease control group and MECS -100 Mg/kg treated group. Treatment with MECS (200 & 400 mg/kg) shown lower level of MDA as shown in figure 13.

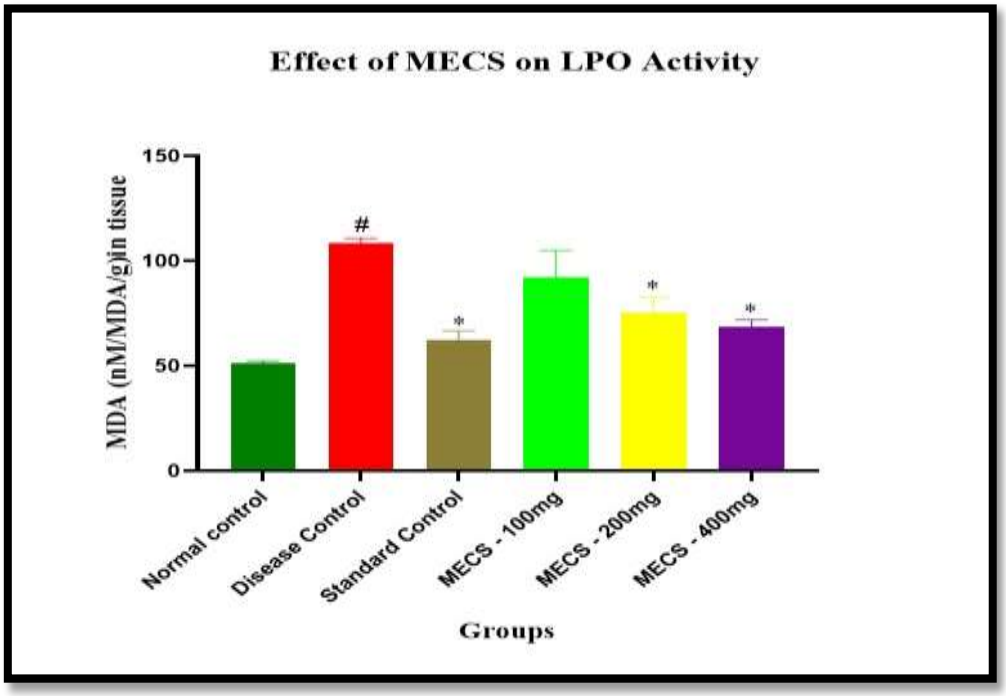
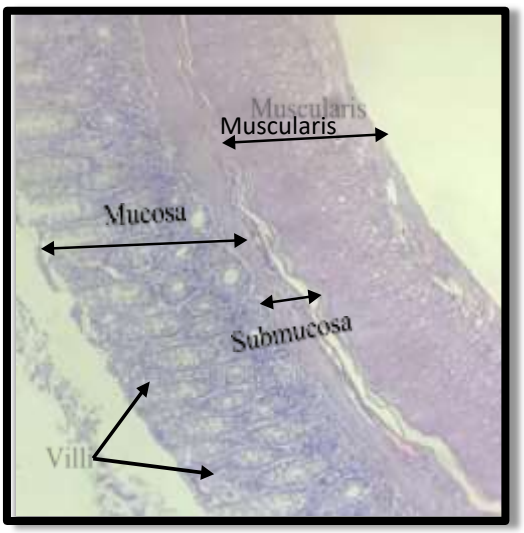


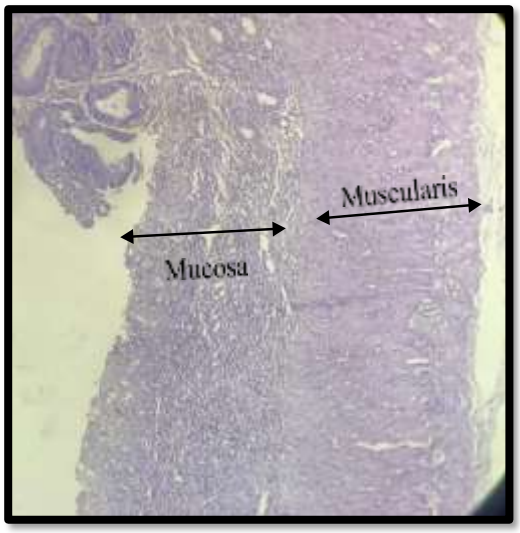
*Figure 13 Effect of Methanolic extract of roots of Clerodendrum serratum L. on LPO activity in TNBS induced UC rats. All values are represented in Mean ± SEM; n=6. *Significant different from disease control (P<0.05) #Significant different from normal control (P<0.05)*

Histopathology of colon

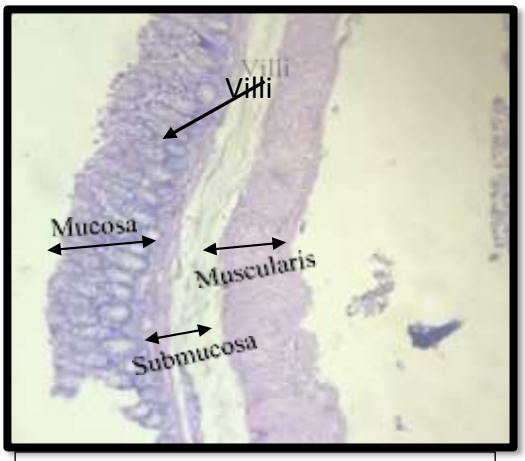
Histopathological analysis shows the typical histopathological changes such as infiltraon of neutrophils, ulcers, distoron of mucosal layer were due to administraon of TNBS in experimental animals.



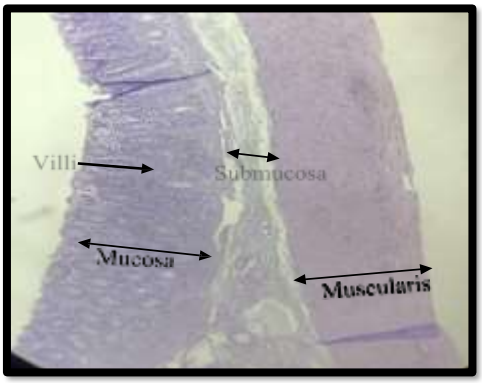
1. NC- Normal histological structure of mucosal layer.



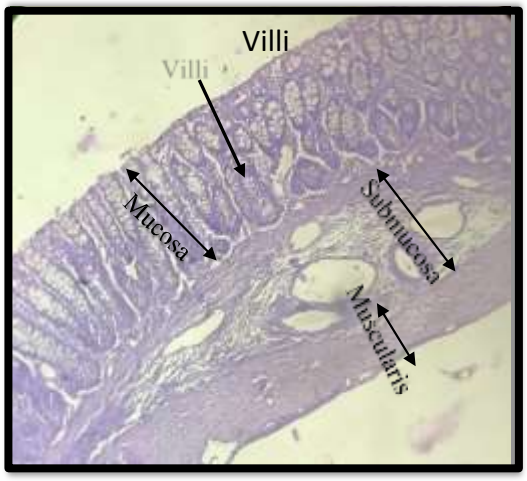
2. DC – Total distortion of mucosa with marked infiltration of neutrophils.

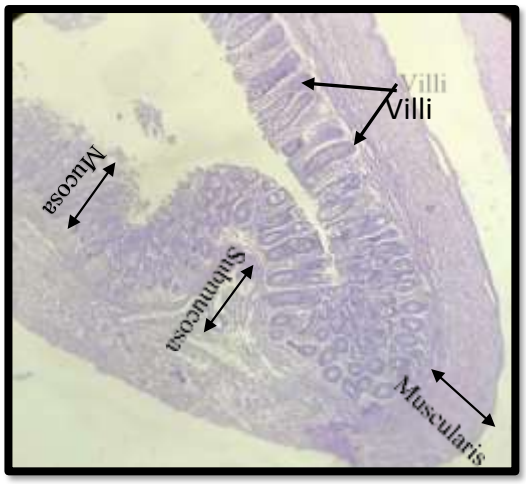


3. SC- Intact mucosal membrane with normal structure architecture.



4. MECS – 100 – Slight dilation of intestinal mucosal glands with mild infiltration of





5. MECS - 200 – Decrease in infiltration of inflammatory cells, near normalization of mucosa.

6. MECS – 400 – Normal structure of mucosa (architecture) with lesser cell infiltration.

Figure 14 Effect MECS on histopathology of colon in TNBS induced ulcerative colitis in rats: 1. Normal Control

2. Disease Control 3. Standard Control 4. MECS -100(100 mg/kg) 5. MECS-200 (200 mg/kg)
Disease control group shown total distoron of mucosal layer with marked infiltraon of neutrophils & ulcers due to TNBS as shown in fig.2.Sulfasalazine group revealed intact mucosal membrane with normal structure of mucosal layer was in fig. 3.Treatment group (MECS100mg/kg) shown mild infiltraon of neutrophils as well as slight dilaon of intesnal mucosal glands.Treatment with MECS (200mg/kg) animals shows decrease in infiltraon of cells while normal structure of mucosa (architecture) with lesser cell infiltraon was observed in MECS (400 mg/kg) treated experimental animals.

4 Discussion

This study explores the therapeuc potenal of Clerodendrum serratum Linn.'s methanolic extract (MECS) in a trinitrobenzene sulfonic acid-induced ulcerave colis model. MECS effecvely migates macroscopic and histological damage in rat intesnes, as evidenced by increased body weight, improved water and food intake, and reduced disease acvity index. Microscopic analysis reveals decreased colonic structural damage, inflammatory infiltraon, and lower levels of proinflammatory cytokines, parcularly TNF-$\alpha\alpha$. MECS also demonstrates anoxidant effects by enhancing superoxide dismutase and catalase levels while reducing lipid peroxidaon and nitric oxide. These findings highlight MECS as a potenal therapeuc agent for ulcerave colis, emphasizing its aninflammatory and anoxidant properes.

5 Conclusion

Treatment with methanolic extract of roots of Clerodendrum Serratum L. (MECS) has significantly decreased colonic damage, Disease acvity index score (DAI), Nitric oxide (NO), Lipid peroxidaon acvity (LPO) and enhanced colon length, colon weight, Sodium dismutase (SOD) and Catalase (CAT) level. It exhibits anoxidant and an - lipid peroxidaon acvity. These findings indicates that the Clerodendrum serratum L. has remarkable an- inflammatory acvity associated with an-oxidant acvity. In conclusion, in this present study all above findings are confirmed that the methanolic extract of roots of Clerodendrum Serratum L. has an -inflammatory effects on TNBS induced ulcerave colis.Further invesgaon is needed to examine the acve principle responsible for the acvity and elucidate the specific mechanism of its therapeuc acvity.

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