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Investigation on Potential of *Leonotis Nepetifolia* in Inflammatory Bowel Disease.

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ABSTRACT :

Inflammatory bowel disease (IBD) is a chronic condition of the intestine with unknown etiology involving multiple immune, genetic and environmental factors. Interested to examine the effect of total extract from *Leonotis nepetifolia*. In the current study, we aimed to verify and explain the potential beneficial effects of *Leonotis nepetifolia* on inflammatory bowel disease (IBD). *Leonotis nepetifolia* extract was administered low and high dose (100, 200 mg/kg/bwt.) through drinking water to IBD wistar rat induced by intrarectal administration of acetic acid. Mesalazine (360mg/kg/bwt) was used as the standard drug for comparison. Food intake, colon length/weight, and disease activity index (DAI) as well as tissue damage scores were evaluated. The inflammatory response to IBD was assessed by measuring the expression of myeloperoxidase (MPO) activity. All acetic acid-induced rats showed typical clinical manifestations of IBD. IBD rats in the low and high dose of extract treatment groups were effective in relieving appetite loss. Low and high dosage of extract significantly reduced the DAI score, whereas colon weight/length ratio was increase and tissue damage scores, *Leonotis nepetifolia* (100 and 200 mg/kg/bwt.) and mesalazine (360mg/kg/bwt) treated groups showed significantly lower score values of macroscopic and microscopic characters when compared with the control group. The MPO activity of negative control group showed significant increase in comparison to normal group (60 versus 159 U g 1 colon). The MPO activity decreased in *Leonotis nepetifola*-treated (100and 200 mg/kg) and mesalazine (360 mg/kg) treated groups compared to control group. The mean percentages of decreases of MPO activity in *Leonotis nepetifola* (100 and 200 mg/kg) and mesalazine treated groups were (69, 67, and 64 U g 1 colon), respectively. The two treatment group shows nearly equal effect as that of the standard drug. All parameters suggest that the 200 mg/kg dose is more effective than the lower dose. Based on the above findings, ethanolic extract of *Leonotis nepetifolia* at 200mg/kg showed significant ($P < 0.05$) inhibition of rat IBD. The ethanolic extract of *Leonotis nepetifolia* 200mg/kg is effective in protecting against acetic acid induced IBD.

Keywords: Inflammatory bowel disease (IBD), *Leonotis nepetifolia*, Mesalazine, Inflammation, Ulcerative Colitis, Crohn's Disease

1. Introduction:

Inflammatory Bowel Disease (IBD) is a chronic intestinal inflammation resulting from complex interactions between the host and microbial factors in genetically susceptible individuals. It encompasses two main conditions: Ulcerative Colitis (UC) and Crohn's Disease (CD), both characterized by inflammation affecting the small and large intestine. The causes of IBD remain unknown, but factors such as bacterial infection, immune system alterations, and genetic variations are believed to contribute. Over the past 50 years, IBD has become more prevalent globally, including in Iran and other Middle Eastern countries. The understanding of IBD has evolved, leading to advancements in its diagnosis, mechanisms, and treatment options. The pathogenesis of IBD involves a combination of triggers, including genetic predisposition, environmental influences, and alterations in the gut microbiota. This interplay can disrupt the balance between pro-inflammatory and anti-inflammatory cytokines, leading to an abnormal immune response in the gastrointestinal tract. Current treatment approaches aim to block the pathological pathways associated with the production and release of pro-inflammatory cytokines. Therapeutic modalities include herbal approaches as well as conventional allopathic treatments such as aminosalicylates, corticosteroids, and immunosuppressive agents like azathioprine, mercaptopurine, or methotrexate. In summary, IBD is a group of autoimmune disorders with unknown etiology, affecting the gastrointestinal tract. Advances in research have provided insights into its pathogenesis and treatment, focusing on addressing the dysregulated immune response and inflammation. Therapeutic strategies involve a combination of genetic, environmental, and microbial factors to manage the complex nature of IBD.[1-5]

According to World Health Organization (WHO), in World Traditional Medicinal Strategy 2014–2023, it is estimated that 80% of the population in developing countries rely on traditional medicine from plant sources. *Leonotis nepetifolia* is one such plant. *Leonotis nepetifolia* is a species of plant in the genus Leonotis and family *Lamiaceae* (mint), sometimes referred to as klip dagga, Christmas candlestick, or lion's ear. It has several pharmacological properties antioxidant activity, anti-inflammatory activity, anticonvulsant activity, anticancer activity etc. *Leonotis nepetifolia* is a species of plant in the rubric Leonotis. It's native to tropical Africa and southern India.[6-16]

2. Material and Methods

Selection of plant:

Drug discovery from medicinal plant has evolved to include numerous field of inquiry & use various method of analysis. The process typically done with a Taxonomist, ethno botanist, ethno Pharmacologist or plant ecologist who identifies the plant of interest. Collection may involve species with known biological activity for which active compounds have not been isolated by solvent. According to the intensive literature survey, *Leonotis nepetifolia* was used for the present study.[17]

Collection, identification and authentication of Plant material:

The plant of *Leonotis nepetifolia* was collected from local areas of Indore and the plant was authenticated by Dr. S.N. Dwivedi A.P.S. College Rewa. Voucher Specimen Number: J/Bot./2023-0131CSWP.

Preparation of extracts:

A Soxhlet extractor was used to carry out the extraction. The finely ground, air-dried whole plant of *Leonotis nepetifolia* were subjected to a 48-hour soxhlation process, which involved a hot extraction utilizing ethanol as a solvent at a temperature of around 60ºC. The extract was distilled in a porcelain evaporating dish and dried over a boiling water bath to produce a dark brown, semi-solid material.[17]

Phytochemical screening:

Alkaloids, Flavonoids, Saponins, Tannins, Proteins and Terpenoids were the main groups of phytochemicals that were qualitatively assessed in the ethanolic extract of *Leonotis nepetifolia.*

Experimental Animals:

Male Wistar rats, weighing 150-180g were taken for study. Then all the animals were kept in the animal house of Swami Vivekananda College, Indore. The animals were kept in the cages at 24±2C, humidity: 50% ± 5% following 12 hours light and 12 hours dark cycle, allow them, free access to water ad libitum and food. Prior to being utilized in the research, the animals were given a week to acclimatize. The experimental protocol was approved by the Institutional Animal Ethical Committee of our institute. (Approval No: IAEC/SVCP/2023/01) and were strictly in accordance with the norms of CPCSEA.

Acute oral toxicity:

As per the literature, the ethanolic extract of *Leonotis nepetifolia* plant was found to be safe when administered at a dosage of 100mg/kg and 200 mg/kg of body weight. No mortality was observed at both doses. Therefore, it was reported that the LD_{50} of the plant extract is 3.8 g/kg of body weight.[17, 21]

Experimental design:

Table no. 1 A total numbers of 20 healthy male wistar rats were divided into 5 groups each:

S. No.	Groups	No. of Animals
1.	Vehicle Controlled Group	2
2.	Negative Control Group (IBD induced)	3
3.	Low dose of extract (100mg/kg) (17)	5
4.	High dose of extract (200 mg/kg) (17)	5
5.	Standard Group Mesalazine (360mg/kg) (18)	5
	Total	**20**

Procedure:

The rats were divided into five different groups. Group1 has served as Vehicle control group. rats were treated with normal saline for six consecutive days. Group 3 rats were treated with low dose (100/mg/kg/bwt) and group 4 rats were treated with high dose (200mh/kg/bwt) of *Leonotis nepetifolia* plant extract. Group 5 rats were treated with standard drug Masalazine (360mg/kg/bwt). These entire drugs were given orally for six consecutive days. After one day fasting, except Group 1 rats in all other experimental rats group 2, group 3 group 4 group 5 were induced inflammatory bowel disease with single dose of 2 ml of 3 % acetic acid by using polyethylene tube of 2 mm in diameter through the rectum into colon region up to 8 cm. The rats were kept in supine trendelenburg position for 30 sec to hinder the intracolonic instillate leakage. For the confirmation of colitis. Rats were anesthetized with 3% of sodium pentobarbital (50mg/kg, i.p) then sacrificed by cervical dislocation after 24 hr of induction and the colon tissue were dissected out for analysis. The dissected colon specimen were washed with ice cold PBS (phosphate buffered saline) (pH 7.2) and stored in 10 % formaline for histopathology and morphologic studies. Treatments were started with the low dose (100mg/kg) and high dose (200mg/kg) of plant extract and standard drug (mesalazine 360mg/kg) 6 h after IBD induction. The dose was administrated via oral gavage. The rat neck was held carefully with the thumb and index fingers and other three fingers were used to hold remaining part of the body. Rat was lifted and held in an upright position and continued for 6 consecutive days. Food intake and stool consistency, rectal bleeding was recorded daily. After 6 days all rats were anesthetized with 3% of sodium

pentobarbital (50mg/kg, i.p) then sacrificed by cervical dislocation and colon tissue were dissected out for analysis. The dissected colon specimen were washed with ice cold PBS (pH 7.2) and stored in 10 % formaline for histopathology, morphologic, and MPO studies.[18, 19, 20]

Evaluation of Inflammatory Bowel Disease:

Disease Activity Index:

Disease Activity Index (DAI) Scores were given based on the percentage of weight loss (1, none; 2, >18%); 3, 12-18%; 4, 6-12; and 5, 1-6% stool consistency (1, normal; 2, watery stool; 3, pasty stool that does not stick to the anus; 4. pasty stool that stuck to the anus; and 5, pasty stool that stuck to the anus), and rectal bleeding (1, hemoccult (-); 2, obvious blood in stool; 3, hemoccult (+); 4, hemoccult (-); and 5, hemoccult).[18]

Change of Colon Length and Colon Weight:

Table no. 2 Change of colon Length and colon weight.

S.no.	Groups	Change of colon Length (cm)	Change of colon Weight
1	Vehicle Control (Normal Control Group)	8.35±0.04	325.84±0.70
2	IBD induced (Negative Control Group)	6.19±0.04	358.24±0.71
3.	Extract (100 mg/kg)	6.45±0.08	355.37± 0.71
4.	Extract (200 mg/kg)	6.66±0.081*	350.34± 0.74*
5.	Standard Drug Group (Mesalazine 360mg/kg/btw	6.73 ±0.10	347.13±0.71

Values are expressed as Mean ± S.E.M. (n=5), Data are expressed as mean ± SEM and results were analyzed by ANOVA using Tukey's multiple comparison test: Significance at $P < 0.05$ Vs control.

Assay of Colon MPO Activity:

Colonic samples were sliced on ice and homogenized in 10 ml of ice-cold 50 mM potassium phosphate buffer (pH 6.0), which also contained 10 mM EDTA and 0.5% HETAB, in order to measure MPO activity. Following sonication, the homogenates were centrifuged at 12,000 g for 20 minutes. Spectrophotometric measurement of MPO activity was performed as follows: 2.9 ml of 50 mM phosphate buffer containing 0.167 mg ml^{-1} O-dianisidine hydrochloride and 0.0005% H_2O_2 was mixed with 0.1 ml of supernatant. Using spectrophotometer, the absorbance change was measured spectrophotometrically at 460 nm. The change in absorbance per minute at room temperature in the final reaction is the definition of one unit of MPO activity. The MPO activity (U g1) is equal to X/weight of the removed tissue, where X is 10 · change in absorbance per minute/volume of supernatant taken in the final reaction.[20]

Assessment of Colonic Damage by Histopathology:

Rats were anesthetized with 3% of sodium pentobarbital (50mg/kg, i.p) on day six. Then after cervical dislocation the entire colon was removed and the length was measured. Also, colons were cut longitudinally along the mesentery, washed with ice-cold PBS (potassium phosphate buffer) (pH 7.2), and counted. Macroscopic damage was scored with variations as follows 1, no ulcers and inflammation 2. mucosal hyperemia and edema 3. ulceration without mucosal hyperemia and edema, 3, single ulceration and mucosal inflammation; 5, further ulceration and mucosal inflammation; and each group, three representative samples were named, fixed in 10% formalin and stained with hematoxylin and eosin (HE, submittal for examination). The histological scores were assessed. Scores were as follows for Vehicle control (1), no colonic tissue damage; for negative control (2), inflammatory cell infiltration, loss of goblet cells, vascular proliferation, and bowel wall thickening with an invaded muscular layer; for low dose of extract (3), , medium level of inflammatory cell infiltration, crypt damage, bowel wall thickening without invasion of the muscular layer; for high dose of extract (4), low level of inflammatory cell infiltration, intestinal villus arranged neatly for standard drug treated (5), low level of inflammatory cell infiltration, intestinal villus arranged neatly.[18]

Figure no. Vehicle Control Group; 2. Induced IBD (Negative control); 3. Induced IBD + *L.nepetifolia* (100mg/kg)

4. Induced IBD + *L.nepetifoila* (200mg/kg); 5. Induced IBD+ Masalazine (360mg/kg)

Statistical analysis:

The findings are given as mean ± S. E. M. One way analysis of variance (ANOVA) and the Turkey HSD test were used for the statistical analysis. The results were compared with those of the standard group and the normal control groups. Differences with values of ($P < 0.05$) were deemed significant for all tests.

3. Results:

Extraction:

The yield of the ethanolic extract of *Leonotis nepetifolia* was 25% w/w.

Qualitative phytochemical analysis of *Leonotis nepetifolia* extract:

The ethanolic extract of whole plant of *Leonotis nepetifolia* includes Alkaloids, Flavonoids, Saponins, Tannins, Proteins and Terpenoids, according to the qualitative phytochemical study.

General Physiological Features:

General physiological features of rats, including body weight, food intake, DAI scores, colon weight and colon length were documented. Rats in the negative control group (IBD induced) showed significant loss in weight and appetite when compared with rats in the vehicle control group ($p < 0.05$). IBD rats also displayed higher DAI scores that were associated with higher incidences of diarrhoea, rectal bleeding, and increased colon weight/length ratio. High and low dose of extract significantly prevented weight and appetite loss in IBD rats ($p < 0.05$). Compared with rats in the control group, the DAI scores of rats in all treatment groups were significantly decreased. In addition, treatment with high and low dose of extract and standard drug treated was effective in reducing colon weight/length ratio.

Effect of Leonotis *nepetifolia* extract on colon length and colon weight:

Acetic acid instillation is associated with marked decrease in the colon length and increase in the colon weight; indicating colonic inflammation. As evidenced from the (Table no.2) colon length was decreased significantly and colon weight slightly increased.

Colon Myeloperoxidase Activity Changes:

The changes in the activity of MPO in bowel homogenates of treated animals is shown in (table no. 3) The changes in the activity of MPO in bowel homogenates of treated animals is shown in (table no.3) The MPO activity of Negative control group (60) showed significant increase in comparison to normal group (159). The MPO activity decreased in *Leonotis nepetifola*-treated (100and 200 mg/kg) and mesalazine (360 mg/kg) treated groups and are comparable to vehicle control group values. The mean percentages of decreases of MPO activity in *Leonotis nepetifola* (100 and 200 mg/kg) and mesalazine treated groups were 69, 67, and 64, respectively.

Table no.3: Assessment of MPO Activity:

S. No.	Groups	Mean ± Sem
1	Vehicle control (normal control group)	60±0.4
2	IBD induced (negative control group)	159±0.4
3	Low Dose of extract (100mg/kg/btw)	69±0.70
4	High Dose of extract (200mg/kg/btw)	67±0.70*
5	Standard Drug Treated Group	64±0.70

Data are expressed as mean ± SEM and results were analysed by ANOVA using Tukey's multiple comparison test: Significance at *$p<0.05$ Vs control.

Macroscopic and Microscopic Assessment of the Colon:

Macroscopic and microscopic scores of rats in the negative control group were significantly higher when compared to rats in the vehicle control group. Ulceration with mucosal hyperaemia and oedema was observed in rats in the negative control group (IBD induced). Such changes were markedly improved by treatment with high and low dose of extract and standard drug. Histological scoring revealed that all doses of high and low dose of extract and standard drug used in this study were effective in mitigating the severity of tissue lesions as characterized by the low level of inflammatory cell infiltration. Moreover, the intestinal villus arranged neatly and the number of goblet cells increased ($p < 0.05$).

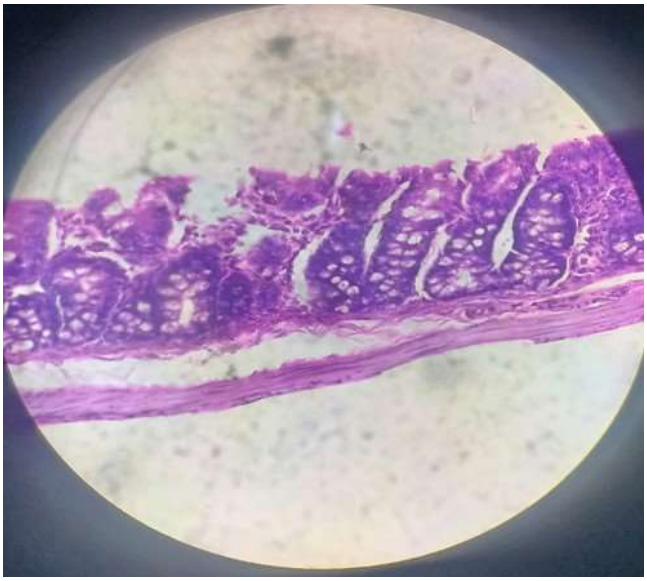

Figure no.2: Vehicle control group macroscopic and histopathological in acetic-acid induced rats

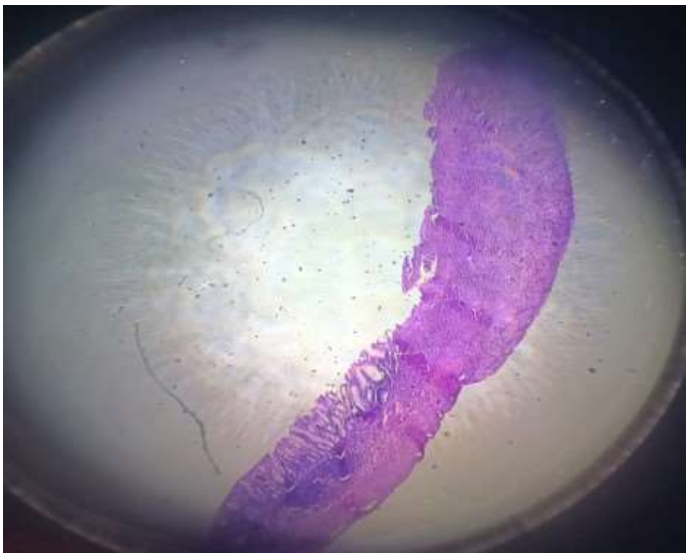

Figure no.3: Macroscopic and histopathological changes in acetic acid-induced rats (Negative Control Group)

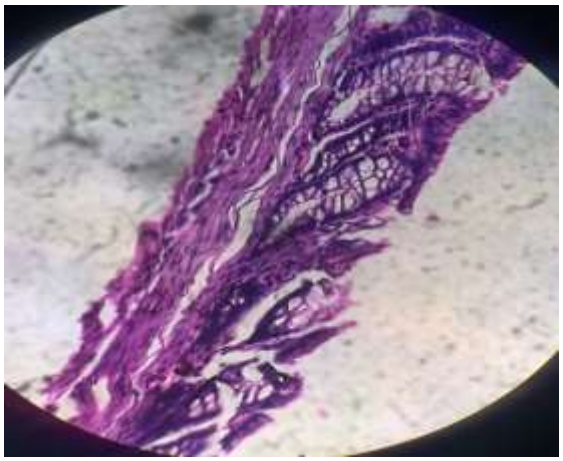

Figure no.4: Effects of *Leonotis nepetifolia* (100mg/kg) administration on macroscopic and histopathological changes in acetic acid-induced rats (Low dose extract group)

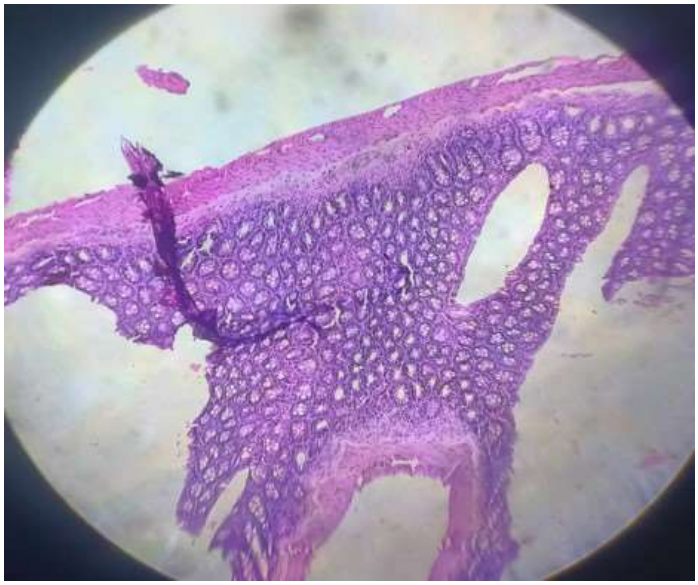

Figure no.5: Effects of Leonotis nepetifolia (200mg/kg) administration on macroscopic and histopathological changes in acetic acid-induced rats (high dose extract group)

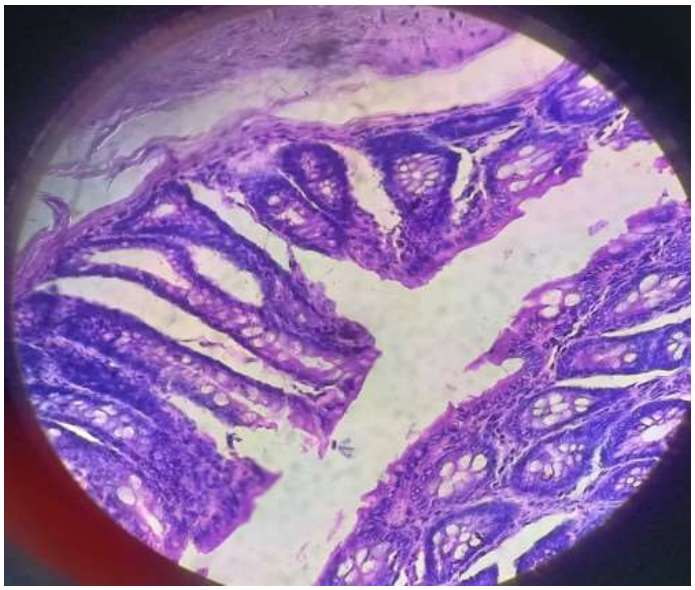

Figure no.6: Effects of Mesalazine (360mg/kg) administration on macroscopic and histopathological changes in acetic acid-induced rats (Standard Drug Group)

4. DISCUSSION

Taking collectively, the data provide very good evidence on benefit of *Leonotis nepetifolia* in experimental model of IBD. Soxhlet apparatus was used to obtain ethanolic extract employing ethanol as a solvent. Successive extraction of *Leonotis nepetifolia* was done. % yield extract was found 25 %. Qualitative chemical examinations of *Leonotis nepetifolia* exposed presence of Alkaloids, Flavonoids, Saponins, Tannins, Proteins and Terpenoids. In this method, a low dose and high dose (100 and 200 mg/kg) of ethanolic extract of *leonotis nepetifolia* was used as test group and the significant activity against inflammatory bowel disease activity was evaluated comparing the test group rats with standard group mesalazine (360 mg/kg) treated wistar rats. Acetic acid-induced IBD has been demonstrated as an ideal rat model for experimental studies on IBD given that it shares several clinical, histological, and immunological features with human IBD disease. Intrarectal administration of acetic acid evoked a colonic inflammation characterized by increased neutrophil infiltration, massive necrosis of mucosal and submucosal layers, submucosal ulceration, increase in vascular dilation, and oedema and also causing a significant effect on colon weight and length. In the present study, It noted that low and high dose of plant extract *Leonotis nepetifolia* (100mg/kg, 200mg/kg) and standard drug mesalazine (360mg/kg) significantly improved general physiological features of treated rats, such as decreasing the extent of the diarrhoea, food intake recovery, colon length, colon weight, and DAI scores. With the help of digital camera, colon sections photographed for the assessment of colon length. Histopathology has been done for assessment of any micro and macroscopic damage of colon. Histological scoring revealed that all doses of high and low dose of extract and standard drug used in this study were effective in mitigating the severity of tissue lesions as

characterized by the low level of inflammatory cell infiltration. Treatment with high dose of extract and Mesalazine dose group impeded the loss in body weight in comparison to low dose of extract animals. As evidenced from the (Table no. 2) colon length was decreased significantly and colon weight slightly increased. The changes in the activity of MPO in bowel homogenates of treated animals are shown in (table no.3). The MPO activity of Negative control group showed significant increase in comparison to normal group (60 versus 159 U g 1 colon). The MPO activity decreased in *Leonotis nepetifola*-treated (100and 200 mg/kg) and mesalazine (360 mg/kg) treated groups compared to control group. The mean percentages of decreases of MPO activity in *Leonotis nepetifola* (100 and 200 mg/kg) and mesalazine treated groups were (69, 67, and 64 U g 1 colon), respectively. Based on the above findings, ethanolic extract of *Leonotis nepetifolia* at 200mg/kg showed significant ($P < 0.05$) inhibition of rat IBD. The ethanolic extract of *Leonotis nepetifolia* 200mg/kg is effective in protecting against acetic acid induced IBD. The two treatment groups show nearly equal effect as that of the standard drug. Hence finding from all parameters suggest that the 200 mg/kg dose is more effective than the lower dose.

5. CONCLUSION

The study thus demonstrated the inflammatory bowel disease activity of ethanolic extract of *Leonotis nepetifolia* herbal extract was found to be effective in the functional recovery of the inflammatory bowel disease. The extracts were effective in mitigating the severity of tissue lesions as characterized by the low level of inflammatory cell infiltration as compared to control group. The result may be attributed to the phytoconstituents such as flavonoids and phenolics present in it which may be due to their in mitigating the severity of tissue lesions and provided scientific evidence to the ethno medicinal futures of herbal formulations. The study concludes that the herbal extract obtained by the dried whole plant of *Leonotis nepetifolia* possess in highly effective in IBD.

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