To evaluate intestinal anti-inflammatory activity of alcoholic extract of seeds of *Centratherum anthelminticum* in experimental model of acute colitis

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ABSTRACT

Ulcerative colitis is an idiopathic chronic relapsing–remitting inflammatory disorder that affects the colon and rectum. The present study was an attempt to investigate *in-vivo* intestinal anti-inflammatory activity by using the seeds of *Centratherum anthelminticum.* Inflammation was successfully produced by intra-rectal administration by using 4% glacial acetic acid in albino Wistar rats via intra rectal route for assessment of acute intestinal anti-inflammatory effect. Pathogenic control animals were only treated with vehicle and simultaneously animals of other groups oral treatment with two doses (500mg/kg & 1000mg/kg b.w) and Prednisolone (2mg/kg b.w) Extract treated of animals significantly reduced in a colon score compared to pathogenic group of animals. Significant decrease in Myeloperoxidation, and Lipidperoxidation as compared to pathogenic group of animals. Extract treatment likely to be responsible for the observed significant intestinal anti-inflammatory effect via anti-oxidant mechanism.

Keywords: Ulcerative colitis, Centrosphere anthelmintic, Acute colitis, Myeloperoxidase, Lipoperoxidation

INTRODUCTION

Ulcerative colitis and Crohn's disease, clinically defined as inflammatory bowel disease (IBD) is chronic inflammatory disease of the colon and rectum. Although etiology of the disease has not yet been clear, possible etiological factors are genetic influences, Immunological abnormalities and environmental agents. In the recent years, role of reactive oxygen species (ROS) in thepathogenesis of IBD has been stressed.[1-3]
Available evidences from the literature strongly suggests cascade of free radical products, subsequent lipid peroxidation reducing cellular anti-oxidant capacity resulting in colonic inflammation. Further, it has been shown that number of inflammatory mediators such as ROS, lysosomal enzymes and products of arachidonic acid metabolism are secreted from granulocytes in the inflamed mucosa in IBD.[1,4-5]IBD is a chronic, remittent or progressive inflammatory conditions that may affect the entire gastrointestinal tract and the colonic mucosa, respectively, and are associated with an increased risk for colon cancer.IBD has long been appreciated to have a genetic basis and likely involves a response of the immune system to some environmental agent(s). The discordance of IBD among monozygotic twins and the development of IBD in immigrants to high prevalence countries and in countries undergoing rapid Westernization also highlight the importance of environmental factors in disease pathogenesis.[6]

Centratherum anthelminticum (L) Kuntze seeds is popular in indigenous system of medicine for treatment of various ailments. Among its several uses its useful uses in curing ulcers and inflammatory swelling, intestinal colic.[7]The current investigation envisages to assess protective effect of alcoholic extract of seeds of *Centratherum anthelminticum* in view of paucity of studies reporting its usefulness in IBD, despite possessing acute and sub-acute anti- inflammatory effect,[8]and reported flavonoid content.[9]

MATERIALS AND METHODS

Plant Material and Extraction

Centratherumant helminticum (L)Kuntze [syn. *Veronicaant helmintica Willd.(Asteraceae)* is known as a *Kalajiri* Seeds were than shade dried coarse powdered and store in air tight container.

Experimental animals

Selection of Test animals: Healthy, Albino Wistar rats (either sex) weighing between 170-200 gms randomly assigned for

Pharmacological investigations

Procedure and experimental protocol of Acute model of colitis.[10] Healthy, albino Wistar rats (either sex) weighing between 170-200 gms were randomly assigned to 5 (Group I-V) groups of 5 animals each (n=5). Group I will be non-colitis control, group II will be colitis control, group III, IV and V will be extract treated (2 doses) and Prednisolone respectively. Experimental animals will be treated with vehicle/extract/Prednisolone, orally for 7 days and colitis was induced after overnight fasting on day 8. On day 9, all animals were sacrificed, colon excised and subjected to biochemical and histo pathological examination to assess colonic damage.

Recording of changes in the body weight

Body weight of all experimental animals was recorded before the beginning of animal experimentation and *post* experimentation, after the last dose.

Dissection, scoring and estimations.

At the end of experimental protocol, abdomen was opened and colon exposed. The distal 8cm of the colon was opened by longitudinal incision. After cleaning colon of fat and mesentry, was cut into three pieces for macroscopic, microscopic assessment of colonic damage and biochemical estimation.

Scoring of inflamed colon of experimental animals of acute model of colitis[10] Macroscopic colonic damage assessment for visible damage as described by Bell *et al*., by an observer blind to treatment protocol.

Biochemical estimations

Colonic tissue sample was be homogenized, centrifuged and in resultant supernatant was subjected to estimation of colonic MPO activity[11] for quantifying inflammation) MDA.[12] as described.

Estimation of Myeloperoxidase [MPO]in the Colon tissue.

MPO activity, marker of neutrophil infiltration was performed as follows: Pieces of inflamed tissues (rat colon- 2 cm/rat ileum-2 cm) was rinsed with ice-cold saline, blotted dry, weighed and excised. Minced tissue was homogenized in 10 volumes of ice-cold potassium phosphate buffer (pH7.4), using tissue homogenizer. The homogenate was centrifuged at 10000rpmfor

30 min at 4°C. Supernatant collected was mixed with *o*-phenylenediamine (660 mg/ml) in phosphate buffer and 300 mM H_2O_2 was used to initiate the reaction. Absorbance was observed at 492 nm at an interval of 30 seconds for 5 min. One unit of MPO activity is defined as the change in absorbance per minute by 1.0 at room temperature, in the final reaction.

Calculation of MPO activity

$$\text{MPO activity (U/g)} = \frac{X}{\text{Wt. of the piece of tissue taken}}$$

$$\text{Where } X = \frac{10 \times \text{change in absorbance per minute}}{\text{Volume of supernatant taken in the final reaction}}$$

Estimation of lipid peroxidation in the Colon tissue.

The level of lipid peroxides was estimated by Thio barbituric acid reaction method as described by Ohkawa*et al.*[4]Briefly, to 0.2 ml of test sample, 0.2 ml of SDS, 1.5 ml of acetic acid and 1.5 ml of TBA were added. The mixture was made up to 4 ml with water and then heated in a water bath at 95°C for 60 minutes. After cooling, 1 ml of water and 5 ml of n-butanol /pyridine mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the organic layer was taken and its absorbance was read at 532 nm. The level of lipid peroxides was expressed as n moles of MDA released/ g wet tissue.

Reagents	Sample	Blank
SDS	0.2 ml	0.2 ml
Supernatant	0.2 ml	……
DDW	1.6ml	1.8 ml
Acetic acid	1.5 ml	1.5ml
TBA	1.5 ml	1.5ml
n-butanol/pyridinemix	5ml	5ml

$$\text{Concentration of MDA} = \frac{\text{Absorbance at532 nm}}{\text{Lx?}} \times D$$

Where,
L:lightpath (1cm).

?:Extinction coefficient1.56x105M-1.Cm-1.

$$D: \text{dilution factor} = \frac{\text{Total volume (10ml)}}{\text{Volume of the sample (0.2ml)}}$$

Statistical Analysis

Allvalueswereexpressedasmean±SEMandsubjectedtostatisticalanalysisnamely, one-way ANOVA followed by multiple comparison tests to determine the level of significance. Values of P <0.5 and less was considered statistically significant.

RESULT

Acute Colitis Model: Effect of extract treatment on the body weight of animals.

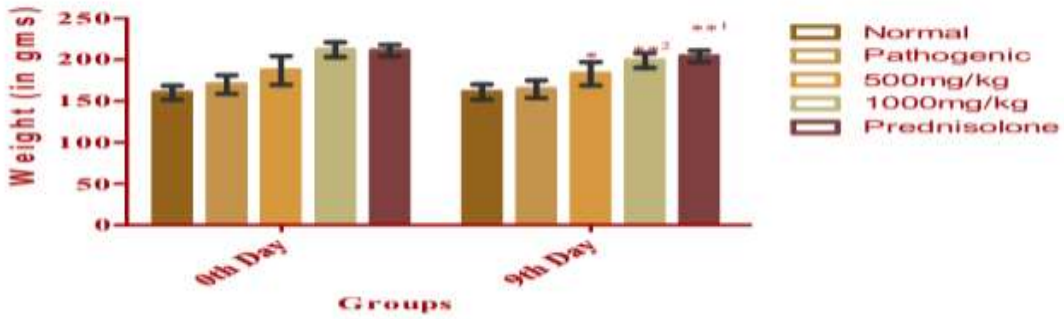
Effects of increasing doses of extract on the bodyweight of animals are as shown in the table-1 and represented as figure-1. No significant difference between various groups of animals exists on day zero. All experimental animals recorded reduction in body weight at the end of study compared to weight on day zero. By day 9th significant difference between body weights of treatment groups compared to vehicle treated pathogenic groups of animals was observed.

Table1: Body weight of experimental animals before and after test protocol.

Group	Treatment	Weight of animals [ingrams]	
		Day0	Day9
I	-	170.0±8.36	171.0±9.13
II	Vehicle	170.0±11.40	164.2±10.54
III	Extract Dose(500mg/kg)	187.0±17.44	183.0±14.11*
IV	Extract Dose(1000mg/kg)	212.0±9.02	199.0±8.86**^{2}
V	Prednisolone (2mg/kg)	211.0±6.78	204.0±6.96^{**1}

All value are mean±SEM of n=5. One way ANOVA followed by Student's Newman-Keuls MultipleComparisonTest.**^{1}P<0.01VsPathogeniconday9,**^{2}P<0.01VsPathogeniconday9,*P < 0.01Vs Pathogenic on day 9

Figure 1: Body weight of experimental animals before and after test protocol.



All value are mean±SEM of n=5. One way ANOVA followed by Student's Newman-Keuls Multiple Comparison Test.
[1]P<0.01VsPathogeniconday9,[2]P<0.01VsPathogeniconday9,*P<0.01Vs Pathogenic on day 9.

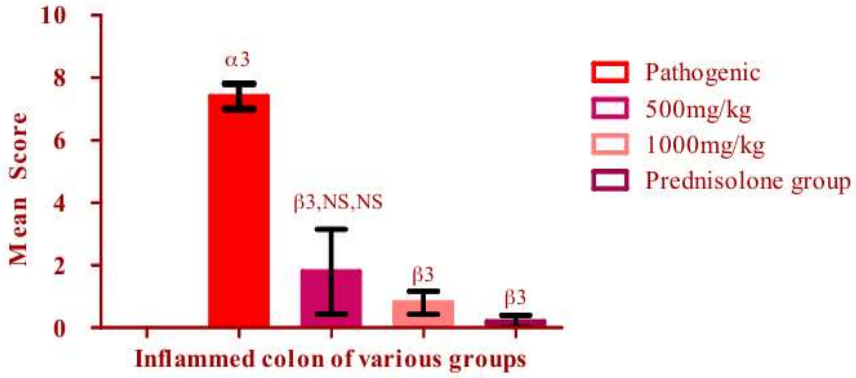
Effect of extract treatment on histopathological changes/macroscopic changes of inflamed colon are shown in table-2 and represented as a figure-2. Significant (P<0.001) increase in colon score was recorded by pathogenic group of animals compared to untreated normal group of animals. Prednisolone group of animals recorded significant (P<0.001) reduction in colon score, compared to pathogenic control group of animals. Extract treated (500mg/kg and 1000mg/kg) animals recorded significant (P<0.001) reduction in colon score compare to pathogenic control group of animals. No significant difference in the colon score was observed in extract treated animals compare to Prednisolone treated group of animals. The colon score was found to be reduced in the dose dependant manner.

Table2: Effect of extract treatment on histopathological changes [Macroscopic scoring for inflammation]

Group	Treatment	Scoring of Colon
I	-	0.00±0.00
II	Vehicle	$7.40\pm0.40^{\alpha3}$
III	Extract Dose(500mg/kg)	$1.80\pm1.35^{NS,NS,\beta3}$
IV	Extract Dose(1000mg/kg)	$0.80\pm o.37^{NS,\beta3}$
V	Prednisolone(2mg/kg)	$0.20\pm0.20^{\beta3}$

All value are mean ± SEM of n=5. One wayANOVA Followed by Students Newman-Keuls Multiple Comparison Test. α3=P<0.001 Vs Normal, β3=P<0.001VsPathogenic, NS **(Non-Significant)** Vs Prednisolone, NS Vs D2 (1000mg/kg).

Figure 2: Effect of extract treatment on histopathological changes [Macroscopic scoring for inflammation.



All value are mean ± SEM of n=5 One way ANOVA Followed by Students's Newman-Keuls Multiple comparison Test.α3=P<0.001 Vs Normal, β3=P<0.001 Vs Pathogenic, NS Vs Prednisolone, NS Vs D2 (1000mg/kg).

Effect of extract treatment on histopathological changes [Macroscopic scoring for inflammation]

				
Figure-1	**Figure-2**	**Figure-3**	**Figure-4**	**Figure-5**
Normal treated	Pathogenic control	Extract treated (500mg/kg)	Extract treated (1000mg/kg)	Prednisolone treated

Effect of extract treatment on MPO activity

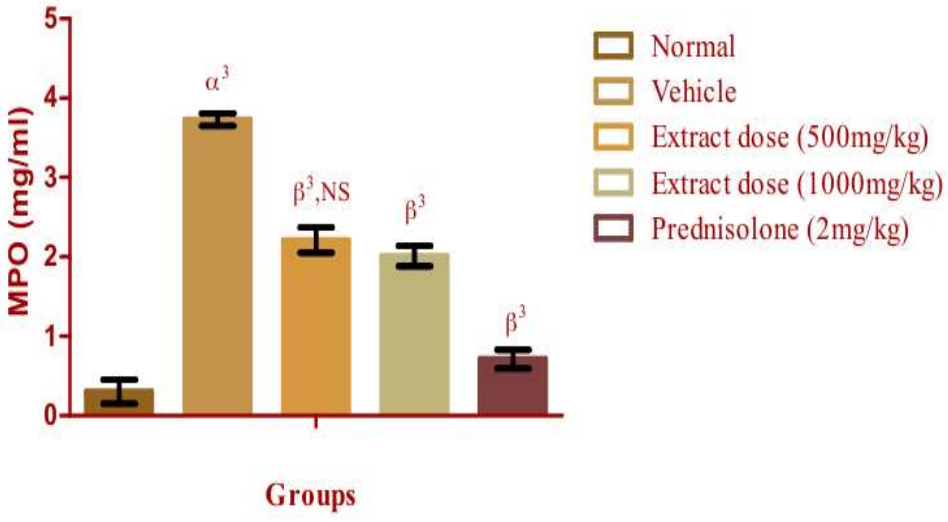
Effect of extract treatment on MPO activity are as shown in table-3 and represent figure- 3. Significant increase in MPO was recorded by vehicle treated pathogenic control group of animals compared to normal group of animals (P<0.001). Significant (P<0.001) reduction in MPO was recorded by extract treated (500mg/kg and1000mg/kg) and Prednisolone (2mg/kg) as compared to vehicle treated pathogenic control group of animals in a dose dependant manner.

Table3: Effect of extract treatment on Myeloperoxidase

Group	Treatment	MPO(mg/ml))
I	-	0.302±0.15
II	Vehicle	$3.730\pm0.08^{\alpha 3}$
III	Extract Dose(500mg/kg)	$2.212\pm0.16^{NS,\beta 3}$
IV	Extract Dose(1000mg/kg)	$2.011\pm0.13^{\beta 3}$
V	Prednisolone(2mg/kg)	$0.713\pm0.12^{\beta 3}$

All value are mean ± SEM of n=5 One way ANOVA Followed by Students's Newman-Keuls Multiple Comparison Test. α^3 P<0.001 Vs Normal control, β^3P<0.001 Vs Pathogenic control, NS Vs D2 (1000mg/kg).

Figure3: Effect of extract treatment on MPO Value.



All value are mean ± SEM of n=5 One way ANOVA Followed by Students's Newman-Keuls Multiple Comparison Test. α^3 P<0.001 Vs Normal control, β^3P<0.001 Vs Pathogenic control, NS Vs D2 (1000mg/kg).

Effect of extract treatment on Lipid Peroxidation (LPO).

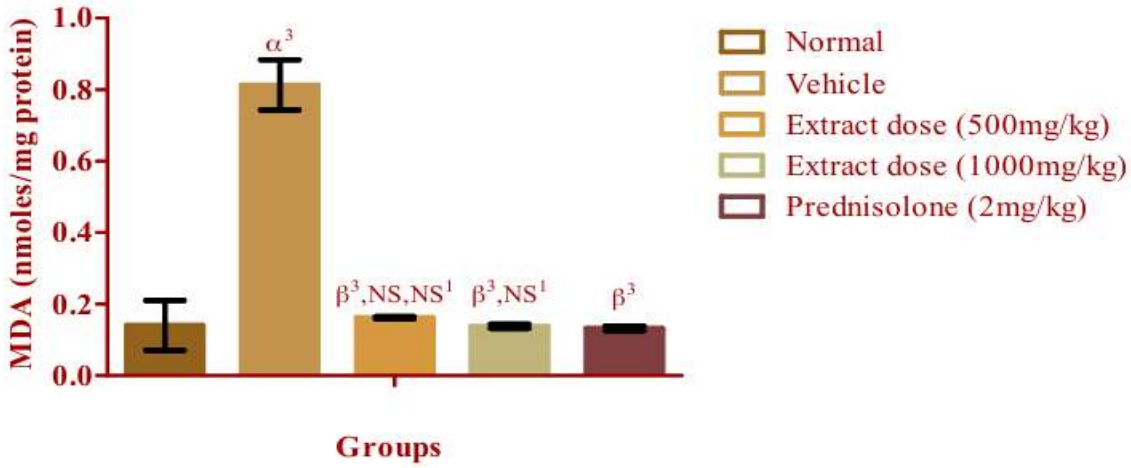
Effect of extract treatment on lipid peroxidation (LPO) are shown in table-4 and represented in figure-4. Significant increase in lipid peroxidation was recorded by vehicle treated pathogenic control group of animals compared to normal group of animals (P<0.001). Significant decrease in lipid peroxidation was recorded by extract treated (500mg/kg and 1000mg/kg) and Prednisolone (2mg/kg) as compared to vehicle treated pathogenic control group of animals. However, significantly higher lipid peroxidation was recorded by extract treated (500mg/kg) extract treated animals compared to Prednisolone treated group ofanimals. Values of lipid peroxidation between extract treated (1000mg/kg) and Prednisolone (2mg/kg) treated animals did not significantly differ. A dose dependant reduction in the magnitude of lipid peroxidation was observed.

Table4: Effect of extract treatment on **lipid peroxidation.**

Group	Treatment	Malondialdehyde (nmoles/mgprotein)
I	-	0.140±0.007
II	Vehicle	$0.812\pm0.071^{\alpha 3}$
III	Extract Dose(500mg/kg)	$0.162\pm0.003^{\beta 3,NS,NS1}$
IV	Extract Dose(1000mg/kg)	$0.138\pm0.006^{\beta 3,NS1}$
V	Prednisolone(2mg/kg)	$0.132\pm0.007^{\beta 3}$

All value are mean ± SEM of n=5 One way ANOVA Followed by Students's Newman-Keuls Multiple Comparison Test. α^3 P<0.001 Vs normal control, β^3 P<0.001 Vs Pathogenic control, NS Vs D2 (1000mg/kg) treated, NS^1 Vs Prednisolone treated.

Figure4: Effect of extract treatment on MDA Value.



All value are mean ± SEM of n=5 One way ANOVA Followed by Students's Newman-Keuls Multiple Comparison Test. α^3 P<0.001 Vs normal control, β^3 P<0.001 Vs Pathogenic control, NS Vs D2 (1000mg/kg) treated, NS^1 Vs Prednisolone treated.

REFERENCES

1. Buffinton GD, Doe WF. Depleted mucosal antioxidant defences in inflammatory bowel disease. Free Radic Bio Med 1995; 19: 911-8.
2. Koch TR, Yuan LX, Stryker SJ, Ratliff P, Tel ford GL, Opara EC. Total antioxidant capacity of colon in patients with chronic ulcerative colitis. DigDisSci2000; 45:1814-9.
3. Mulder TP, Verspaget HW,Janssens AR, deBruin PA, Pena AS, Lamers CB. Decrease in two intestinal copper/Zn containing proteins with antioxidant function in inflammatory bowel disease. Gut 1991; 32: 1146-50.
4. Fantone JC, Ward PA. Role of oxygen derived free radicals and metabolites in leucocyte- dependent inflammatory reactions.AmJPath1982;107:395-418.
5. FloheL, Beckmann R, Giertz H, Loschen G. Oxygen-centred free radicals as mediators of Inflammation in oxidative stress, SiesH(ed)London,AcademicPress1985;PP403-36.
6. KaserA,Zeissig S,Blumberg R S. Inflammatory Bowel Disease. Annu Rev Immunol 2010;28:573–621.
7. Kirtikar KR, Basu B D.IndianMedicinal Plants,Dehradun; International book distributers1975.
8. Ashok P, Koti BC, Thippeswamy AH, Tikare VP, Dabadi P, Viswanathswamy AH. Evaluation of anti-inflammatory activity of *Centratherum anthelminticum (L)* Seed. Indian J Pharm Sci 2010; 72: 697-703.
9. Tian G^1, Zhang U, Zhang T,Yang F, ItoY.Separation of flavonoids from the seeds of *Vernonia anthelmintica (L) Willd* by high speed counters current chromatography. J Chromatogr A 2004; 1049: 219-22.
10. Bell CJ,Gall DG,Wallace GL.Disruption of colonic electrolyte transport in experimental colitis. AmJ Physiol1995;268:G622-30.
11. Krawisz JE, Sharon P,Stenson WF.Qualitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. Gastroenterology 1984; 87: 1344-50.
12. Ohkawa H, Ohishi N, Yagi K. Assay of lipidperoxidase in animal tissues by thiobarbituricacid reaction. AnalBiochem1979;95:351-8.