INTERNATIONAL JOURNAL OF PHYTOTHERAPY RESEARCH
ISSN 2278 – 5701

Original Research Article

PROTECTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *ALLOPHYLUS SERRATUS* (WHOLE PLANT) AGAINST DNBS-INUDCED COLITIS IN RATS

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ABSTRACT:
Ulcerative Colitis involves a chronic inflammation of large intestine, also referred to as colon. The colon is a part of digestive system where waste material is stored. Persons with ulcerative colitis experience inflammation and ulcers of the inner lining of their colon that lead to diarrhea, rectal bleeding and abdominal pain. Ulcerative Colitis is closely related to Crohns's disease; they are commonly referred to as 'Inflammatory Bowel Disease' (IBD). *Allophylus serratus* has been used as anti-inflammatory and diarrhea, to treat wounds and incase of osteoporosis. The present study was to evaluate the anti-colitis activity of Ethanolic extract of *Allophylus serratus* (EEAS) against DNBS induced colitis in rats. According to the present study the animals assigned were divided into four groups six animals in each group. Group I is normal, Group II is DNBS induced, Group III is treated with EEAS 200mg/kg and Group IV is treated with EEAS 400mg/kg. The rats of groups III, IV were pre-treated with the extract for 7 days, on the 8th day the animals of Group III, IV, V were given DNBS followed by administration of extracts for 3 days. At the end of study the animals were sacrificed and bio chemical parameters like MDA, MPO, GSH , NO were measured. Hence the present study showed evidence that the ethanolic extract of might possess anti colitis activity.

KEYWORDS: *Allophylus serratus*, Di-nitro benzene sulfonic acid (DNBS), Colitis, EEAS (Ethanolic extract of *Allophylus serratus*)

INTRODUCTION

Ulcerative colitis and Crohn's disease are chronic relapsing, immunologically mediated disorders that are collectively referred to as inflammatory bowel diseases (IBD). Etiology and pathogenesis remains obscure, although environmental factors, in combination with genetic factors, are suggested to be involved in its pathogenesis [1]. Prolonged or inadequate activation of the intestinal immune system participates in the pathological events of chronic mucosal inflammation [2]. Tumor necrosis factor-α(TNF-α) is a key immunoregulatory cytokine that plays a pivotal role both in experimental and clinical

studies of ulcerative colitis and amplifies the inflammatory response by activating cascade of immune responses[3].

This cytokine stimulates also the production of other cytokines, arachidonic acid metabolites, and proteases [4]. Infiltration of the inflammatory cells, such as neutrophills, in addition to the overproduction of pro-inflammatory cytokines [5] ultimately gives rise to mucosal disruption and ulceration[6]. Infiltered neutrophills, assessed by myeloperoxidase (MPO), are a source of production of cytotoxic reactive oxygen species (ROS). The latter also play a fundamental role in the production of chronic bowel inflammations through their destructive effect on cell macromolecules [7].

EPIDEMIOLOGY

In the western countries the prevalence of the IBD is about 150-200 per 100,000. The incidence of IBD has been increasing rapidly in china recently. Currently the exact causes and mechanisms of IBD are not clear and there is a lack of effective therapy to cure the disease. The mainstream treatment of patients with IBD has been the use of drugs of anti-inflammation, immunomodulation, and antibiotics to relive the symptoms. Steroids and non-steroidal anti-inflammatory drugs are effective for temporarily symptomatic relief. However, drug-induced several side effects have limited their use, particularly for long-term therapy. Therefore IBD has become a worldwide problem and it has been listed as one of the ten most difficult diseases to be treated by WHO.

Patients with these diseases are 10-20 times more likely to get cancer of the colon[8].Ulcerative colitis is treated as an autoimmune disease. Although ulcerative colitis has no known cause, there is a presumed genetic component to susceptibility. The disease may be triggered in a susceptible person by environmental factors. Although dietary modification may reduce the discomfort of a person with the disease, ulcerative colitis is not thought to be caused by dietary factors.

The geographical distribution of the colitis and crohn's disease is similar worldwide, with highest incidence in the United States, Canada, and United Kingdom, and Scandinavia. Higher incidences are seen in northern locations compared to southern locations in Europe and United States.

Age: Ulcerative colitis usually begins before the age of 30. But, it can occur at any age, and some people may not develop the disease until their 50s or 60s

Race or ethnicity: Although whites have the highest risk of the disease, it can occur in any race. If you're of Ashkenazi Jewish descent, your risk is even higher.

Family history: You're at higher risk if you have a close relative, such as a parent, sibling or child, with the disease.

The causes of the colitis is due to the -

Immune system. Some scientists think a virus or bacterium may trigger ulcerative colitis. The digestive tract becomes inflamed when your immune system tries to fight off the invading microorganism (pathogen). It's also possible that inflammation may stem from an autoimmune reaction in which your body mounts an immune response even though no pathogen is present.

Heredity. Because you're more likely to develop ulcerative colitis if you have a parent or sibling with the disease, scientists suspect that genetic makeup may play a contributing

role. However, most people who have ulcerative colitis don't have a family history of this disorder.

The treatment of one cause of colitis, necrotizing enterocolitis (NEC), includes cessation of feedings, nasogastric decompression and I.V fluid resuscitation with attention to electrolytes and acid-base balance.

Antibiotics will be started as soon as as cultures are obtained. Close monitoring with cardio respiratory support is provided as requires surgical therapy is initiated if medical therapy fails

Herbalism, a traditional medicinal or folk medicinal practice based on the use of plants and plant extracts. Herbalism is also known as Phytotherapy. The scope of herbal medicine is extended as a way to learn about potential future medicines [9]. Herbal products are often perceived as safe because they are natural.Natural products drug discovery will be more holistic, personalized and involve wide use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be occurred to the patients and community. In India, in recent years, there is increased research on traditional Ayurvedic herbal medicines, one of the most ancient and yet living traditions practiced widely in developing countries as well as developed countries and has a sound philosophical and experimental basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied.

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. *Allophylus serratus* is a well known plant traditionally it has been used as Anti-Inflammatory; Anti-diarrheal wound healing, Antiulcerogenic, Anorexia, and General Debility etc.

MATERIALS AND METHODS

PLANT MATERIAL

The whole plant of the *Allophylus serratus* kurz was collected from natural habitat of Tamil nadu. The plant was authenticated by Prof. Dr. Santhan. Voucher NO.121/ACNRPL/2012-13

PLANT EXTRACTION

The leaves of *Allophylus Serratus* was washed thoroughly in water, cut in to small pieces and air dried for two weeks at 35-40^{o}C temperature. Extraction was done by using soxhlet apparatus with 70% ethanol (hydro alcoholic) as solvent. The extracts were concentrated under reduced pressure dried and stored at 4^{o} temperature in air tight containers for further studies. The extracts were stored in desiccators and used for further experiments after suspending in the distilled water [10].

Test for Phytochemical analysis

The extracts were analyzed for the presence of Phenols, Flavanoids, Tannins, Saponins and Alkaloids, Glycosides, Steroids, Carbohydrates

Test for Phenols

The Ferric chloride test can be administered by dissolving 15 mg of the unknown compound in 0.5 ml of water or water-alcohol mixture and added 1 to 2 drops

of 1% aqueous ferric chloride solution. Appearance of purple color indicated the presence of Phenols

Test for Alkaloids

0.2 gm of plant extract was weighed and warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragendroff's reagent was added and appearance of orange red precipitates indicated the presence of alkaloids.

Test for Saponins

To 0.2 gm of plant extract, 5 ml of distilled water was added and then heated to boil. Appearance of frothing (appearance of creamy mass of small bubbles), showed the presence of Saponins.

Test for Tannins

Small quantity of plant extract was mixed with water and heated on water bath. The mixture was filtered and Ferric chloride was added to the filtrate. And appeareance of dark green solutions indicated the presence of tannins.

Test for Flavanoids

To 0.2 gm plant extract, dilute Sodium hydroxide and dilute Hydrochloric acid was added and observed for yellow solution that turns colorless indicated the presence of flavanoids

Test for Carbohydrates

Benedict's test – Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, appearance of the reddish brown precipitate showed a positive result for the presence of carbohydrate.

Test for Steroids

Libermann-Burchard test**:** The extract was treated with 3ml of acetic anhydride, few drops of Glacial Acetic acid followed by a drop of concentrated Sulphuric acid. Appearance of bluish green color indicated the presence of steroids.

EXPERIMENTAL ANIMALS

Albino Wistar rats of male sex (200-250g) was collected from King's Institute, Guindy. They were housed in polypropylene cages, with standard rat pellet food and water for several days before the beginning of the experiment with natural light: dark cycle.

CHEMICALS

DNBS(Di nitro benzene sulfonic acid), ethanol, schiff's reagent, hexadecy-trimethyl-ammonium bromide, tetra-methyl benzidine, sodium dodecyl sulfate, acetic acid, hydrochloric acid, thio barbituric acid, griess reagent, nitric oxide, 5,5' dithiobis-(2-nitrobenzoic acid)

INDUCTION OF COLITIS

Colitis was induced using the technique of acid induced colon inflammation, as described by Cuzzocrea *et al*[11]. In fasted rats lightly anesthetized with ether, a catheter was inserted into the colon via the anus until approximately the splenic flexure(8 cm from

the anus).DNBS(25 mg/rat)was dissolved in 50% ethanol. Thereafter, the animals was kept for 15 minutes in a Trendelenburg position to avoid reflux. Six animals were kept as normal. Body weight and food intake of the rats were measured daily throughout the entire experimental period. After colitis induction, the animals was observed for three days. On day four, the animals was sacrificed and abdomen was opened by a midline incision. The colon was removed, freed from surrounding tissues, opened along the anti-mesenteric border, rinsed, weighed and processed for histology.

METHODOLOGY

1. Day 1 to 10 - The animals was housed in the propylene cages and maintained under standard conditions (12 hr light and dark cycles) at 25±3ºc and 35 to 60% humidity
2. Day 11 to 17 – The animals was pre-treated with the different doses of the plant extract for 7 days
3. Day 18 – Induction using DNBS(25mg/rat) dissolve in 50% ethanol
4. Day 19-21 – The animals was treated again with the drug. On 21st day animals were sacrificed and abdomen was opened by mid line incision and the colon was taken for further analysis [12].

Evaluation of the Damaged Lesion Area:

Lesion area was measured as described by Khan [13].In brief; the opened colonic samples were flattened and carefully sandwiched between the two layers of a transparent plastic folder of A4 size.

Histopathology

Colon tissues were fixed in 10% neutral buffered formalin solution and embedded in paraffin .The tissues were then cut into 3µm sections with uniform shape and size, mounted on silane coated glass slides with the help of egg albumin.

Myeloperoxidase (MPO) Activity

Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte (PMN) accumulation was determined as previously determined[14]. Four days after intracolonic injection of DNBS the colon was removed and weighed. The colon was homogenized in a solution containing 0.5% hexadecy -trimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged (REMI C24) for 30 minutes at 20,000 g at 4^0C.An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (1.6 mM) and 0.1 mM H_2O_2 [15]. The rate of change in absorbance was measured spectroscopically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1µmol of peroxide/minute at 37^0C and was expressed in milliunits per 100 gram weight of wet tissue [16].

Malondialdehyde (MDA) measurement

One ml of supernatant was mixed with 0.2ml 4% w/v sodium dodecyl sulphate, 1.5 ml 20% acetic acid in 0.27M hydrochloric acid (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid (TBA) in test tube. The mixture was heated in a water bath at 85^0C for 1 hr. The intensity of pink color developed was measured against the reagent blank at 532 nm. Malondialdehyde was calculated using molar extinction coefficient 1.56*105 M^{-1} cm^{-1} or from standard curve and reported as millimoles per mg of wet tissue [17].

Nitrite Analysis

Accumulated nitrite (NO_2-) in the homogenate was spectroscopically determined based on the Griess reaction[12].The samples (100µl) were based on the Griess reaction. The samples (100µl) were kept at room temperature for 10 min and then NO_2-concentration was determined by the absorbance at 540 nm. The standard curve will be obtained using the known concentrations of sodium nitrate.

Measurement of GSH level in the Colonic Tissue

Colonic tissue was homogenized in ice-cold 125 mmol/1 sodium phosphate buffer with 6.3 mmol/1 EDTA (pH 7.5, 3µl/mg tissue) for 30 s. The crude homogenate was centrifuged at 30,000 g at 4^0C for 30 min. Then, 200µl of 40 g/1 sulfosalicylic acid was added to 100µl of supernatant and allow to stand on ice for 5 min to precipitate protein. The mixture was centrifuged against 5,000 r/min at 4^0C for 10 min. Subsequently 100µl of the de-proteinized supernatant was mixed well with 300µl of 125 mmol/1 sodium phosphate buffer(pH 8),and 2µl of 10 mmol/15,5'-dithiobis-(2-nitrobenzoic acid).The solution was allowed to stand at room temperature for 15 min to develop yellow colour. The absorbance was read against reagent blank at 412 nm in a spectrophotometer. A standard curve of reduced GSH will be used for the calculation of the concentration of GSH in the colonic tissues. The final values were mentioned as nanomole per milligram[18].

STATISTICAL ANALYSIS:

The data obtained from the tests above were analyzed for significance using ANOVA followed by Bonferroni multiple comparison test by the use of Graph pad prism software. Values are expressed as Mean± standard Error

TABLES

TABLE NO 1: EXPERIMENTAL DESIGN

GROUPS	TREATMENT	NUMBER OF ANIMALS(RATS)
GROUP-I	Normal	6
GROUP-II	Control DNBS induced(25mg/rat)	6
GROUP-III	DNBS+EEAS 200mg/kg	6
GROUP-IV	DNBS+EEAS 400mg/kg	6

Table No. 2: Preliminary Phytochemical Screening for EEAS

SL.No.	Phytochemical Tests	Results
1	Alkaloids	Absent
2	Saponins	Present
3	Tannins	Present
4	Flavonoids	Present
5	Phenolic compounds	Present
6	Glycoside	Present
7	Steroids	Present
8	Carbohydrates	Absent

Table No.3: EFFECT OF EEAS ON GENERAL PARAMETERS IN RATS

The effect of EEAS on general parameters showed a significant decrease in the Body weight, Colon weight, Spleen weight and Lesion area when compared to the control group(DNBS administered), which is tabulated in **Table No.3**.

S.no	Groups of Animals	Body weight (g)	Colon weight (g)	Spleen weight (g)	Lesion Area (sq mm)
1	Normal	1.228±0.344	2.083±0.116	0.366±0.061	0.051±0.010
2	DNBS Treated	7.470±0.841**	4.100±0.136**	1.600±0.096**	53.15±1.014*
3	EEAS200mg/kg	4.536±0.422**	3.500±0.103**	1.317±0.079**	35.18±1.261*
4	EEAS400mg/kg	2.733±0.173**	3.017±0.065**	0.616±0.083**	7.250±0.730*

All values are expressed as mean ± SEM (n=6); Data were analyzed by ANOVA followed Bonferroni multiple comparision test. Compared Normal Vs DNBS control: ***p< 0.05*; Compared DNBS control Vs Group III, IV; ***p<0.001* statistically significant.

Table No. 4: EFFECT OF EEAS ON BIOCHEMICAL PARAMETERS

A significant reduction in Malondialdehyde, Myeloperoxidase enzyme compared to control group was observed in a dose dependent manner in groups III and IV Table **No. 4**.

S.No	Treatment	MDA µmole/mg wet tissue	MPO mU/100mg tissue
1	Normal	52.74±1.113	25.42±0.977
2	Control	142.7±2.331**	84.99±1.100**
3	EEAS 200 mg/kg	112.4±2.235*	62.11±1.707**
4	EEAS 400 mg/kg	77.51±2.376*	54.94±0.802**

All values are expressed as mean ± SEM (n=6); Data were analyzed by ANOVA followed by Bonferroni multiple comparision test. All values are expressed as mean ± SEM (n=6); Compared Normal Vs DNBS control: ***p< 0.01*; Compared DNBS control Vs Group III, IV ***p<0.001*; statistically significant

ISSN 2278 – 5701

Table No. 5: EFFECT OF EEAS ON OTHER BIOCHEMICAL PARAMETERS

A significant reduction in nitrite levels and an increase in GSH levels was observed when compared to the control group. Results are tabulated in **Table No. 5.**

S.NO	Treatment	NO (mM/g)	GSH (mM/mg)
1	Normal	5.972±0.409	16.13±0.897
2	Control	130.3±2.657**	4.043±0.336**
3	EEAS 200mg/kg	105.7±1.766***	9.432±0.126**
4	EEAS 400mg/kg	56.18±1.339***	13.26±0.267**

All values are expressed as mean ± SEM (n=6); Data were analyzed by ANOVA followed Bonferroni multiple comparision test. Compared Normal Vs DNBS control: ****p< 0.001*; Compared DNBS control Vs Group III, IV and: ***p<0.05*; statistically significant.

Figure No 1: EFFECT OF EEAS ON BODY WEIGHT

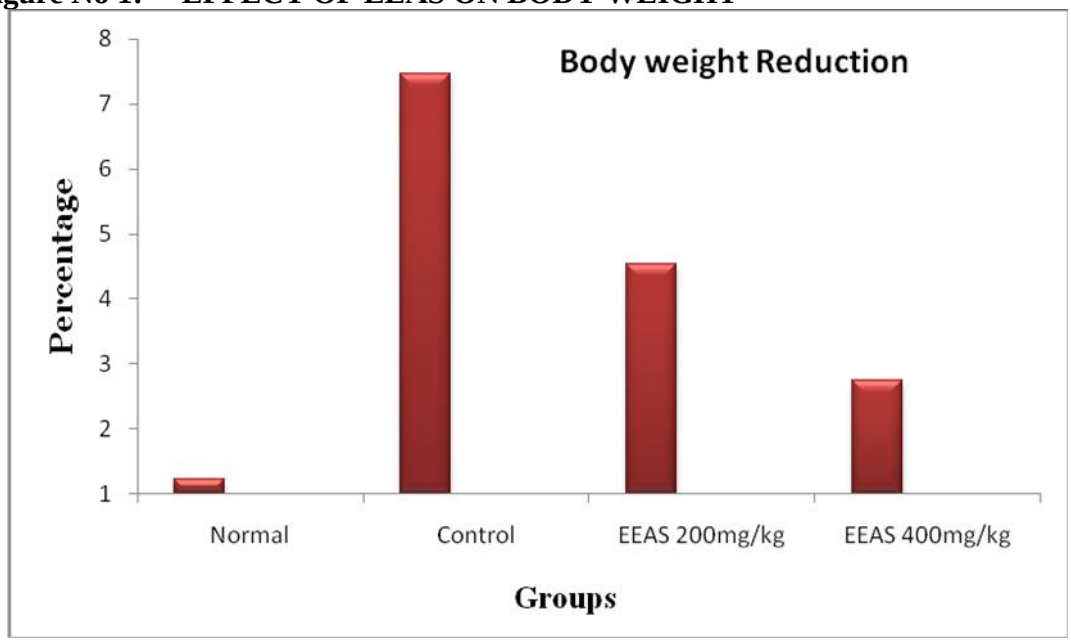


Figure No 2: EFFECT OF EEAS ON COLON AND SPLEEN WEIGHT

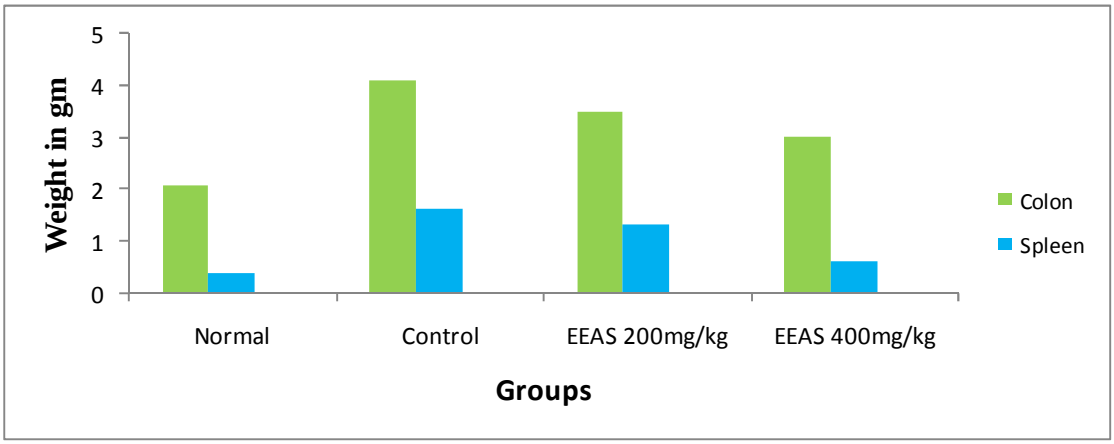


Figure No 3: EFFECT OF EEAS ON LESION AREA

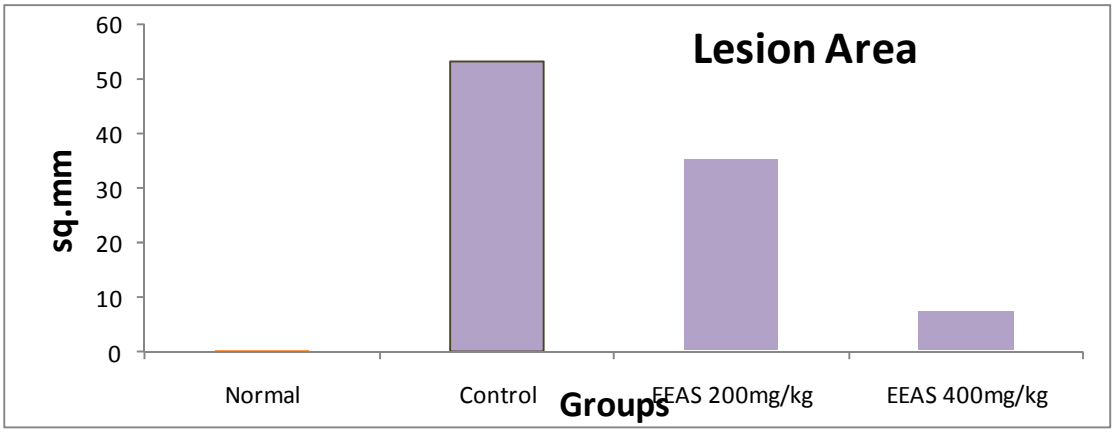


Figure No 4 : EFFECT OF EEAS ON MDA LEVELS

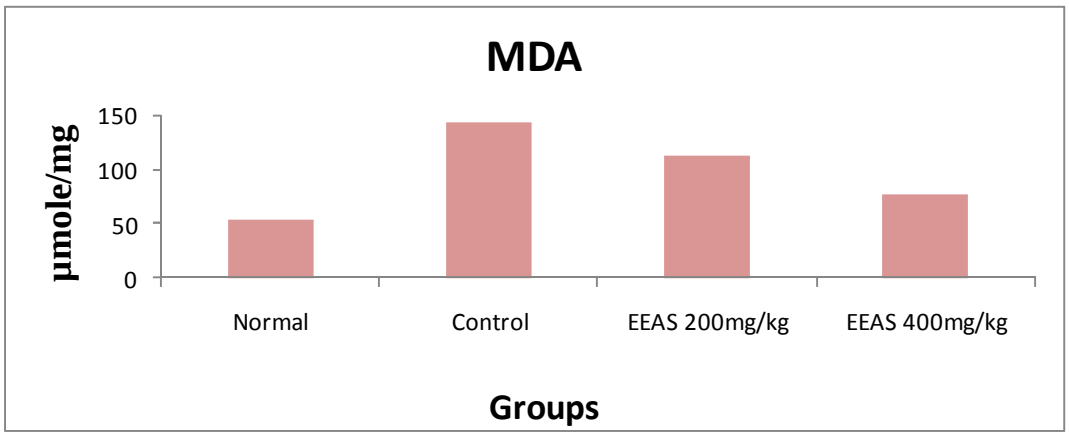


Figure No 5 : EFFECT OF EEAS ON MPO LEVELS

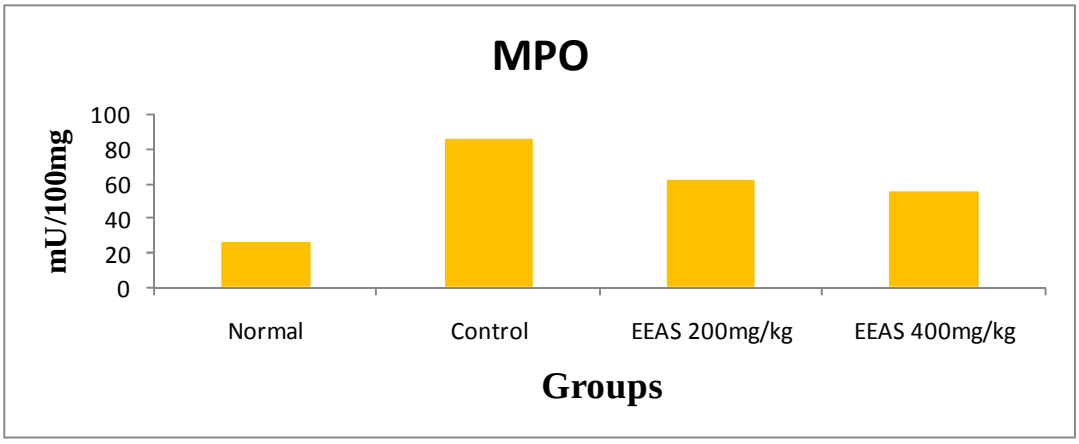


Figure No 6: EFFECT OF EEAS ON NO LEVELS

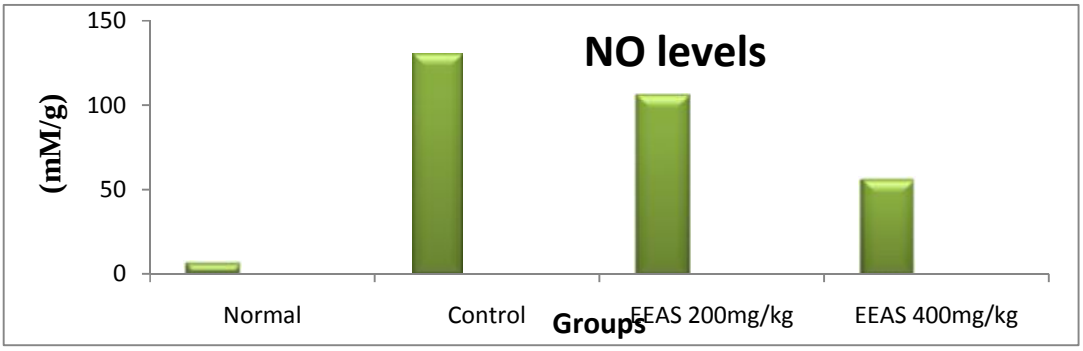
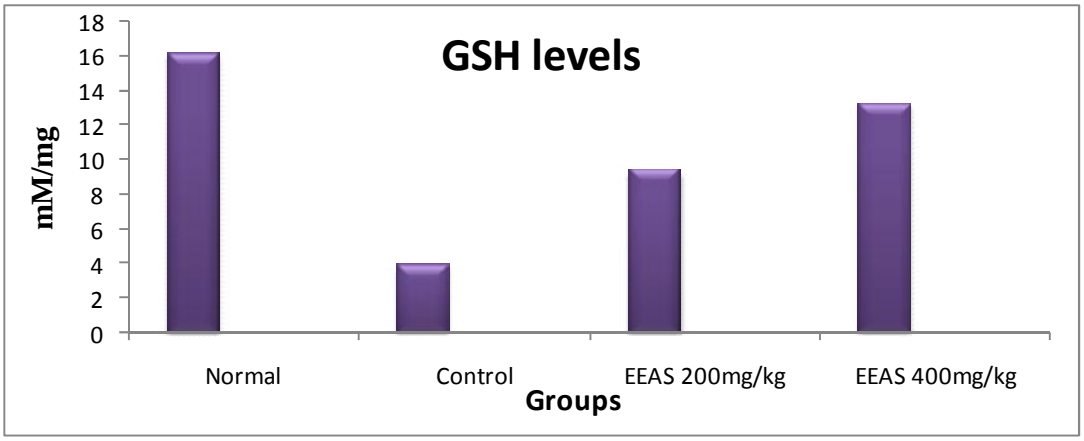


Figure No 7: EFFECT OF EEAS ON GSH LEVELS

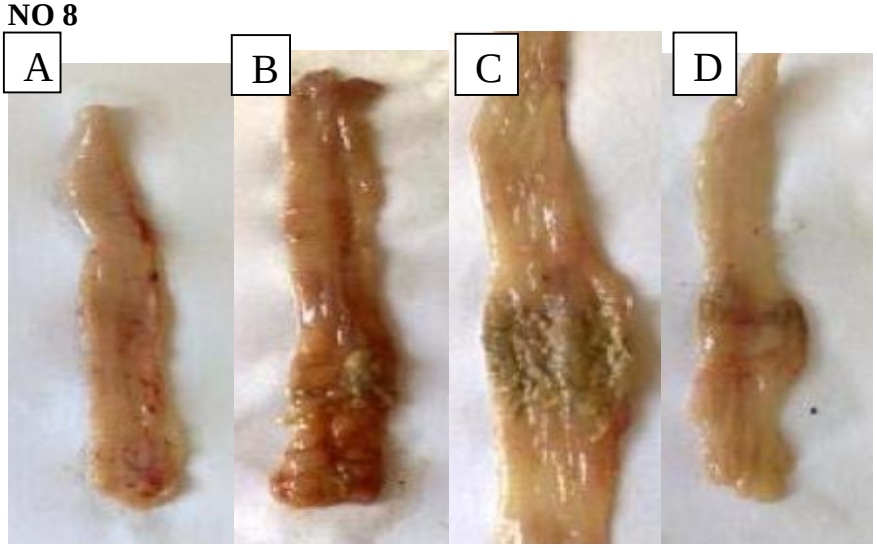


Macroscopic Observation

The colon tissues of respective groups were dissected and observed for ulcerations.

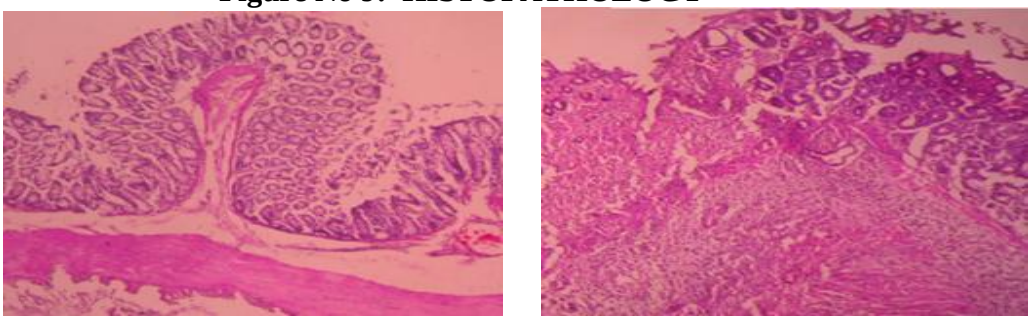
ISSN 2278 – 5701

FIGURE NO 8

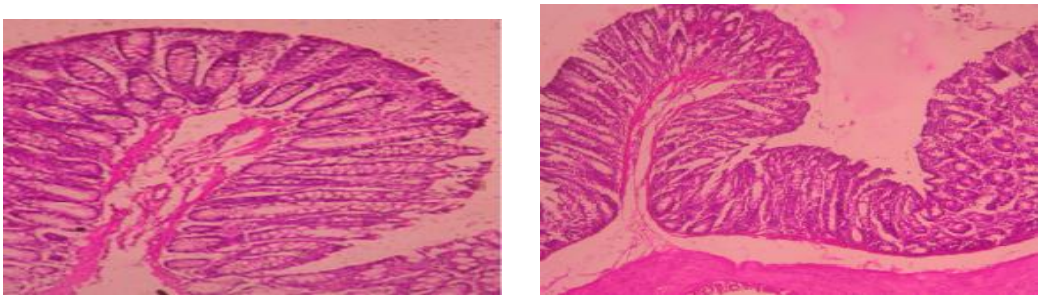


A - Group I: Received Saline solution
B - Group II: Received DNBS 25mg/kg
C - Group III: Received 200mg/kg extract
D - Group IV: Received 400mg/kg extract

Figure No 9: HISTOPATHOLOGY



Normal **DNBS**



EEAS 200mg/kg **EEAS 400mg/kg**

- **In normal group goblets cells are present.**
- **In DNBS group goblet cells are absent.**
- **In EEAS 200mg/kg group goblet cells reformation occurs.**
- **In EEAS 400mg/kg group more improvement in goblet cells reformation.**

DISCUSSION

Ulcerative Colitis involves a chronic inflammation of the person's large intestine, also referred to as their 'colon.' The colon is a part of a person's digestive system where waste material is stored. Persons with ulcerative colitis experience inflammation and ulcers of the inner lining of their colon that lead to diarrhea, rectal bleeding, and abdominal pain**.**

From the previous studies it has been hypothesized that the plant of Allophylus serratus has traditionally used for its anti inflammatory and anti diarrheal properties. Hence the present study was performed to evaluate the claimed traditional use

The present study demonstrated that the ethanolic extract of *Allophylus serratus* prevents tissue damage in rat model of colitis induced by DNBS (di-nitro benzene sulfonic acid) as verified from its effects on macroscopic, histological, and biochemical changes.

The hapten model of colonic inflammation induced by DNBS delivered intra rectally causes a substantial degree of inflammation and tissue injury in the rat colon, resembling human IBD in terms of its various histological features including polymorphonuclear colonic infiltrate and predominant NF-KB dependent Th1 activation.

The results showed that the extract reversed the lost body weight, decreased the gross rectal bleeding and the degree of inflammation, and reduced the histological signs of inflammation such as infiltration by polymorphonuclear leukocytes and multiple erosive lesions.

Preliminary phytochemical studies have been performed and results showed the presence of Saponins, Tannins, Flavanoids, Phenolic compounds, Steroids, Glycosides [19]. Leaves of the plant contain steroidal compounds which are responsible for reducing the ulcerations according to the previous findings it has been reported that β-sterol is mainly useful for reducing the ulcerations. Presence of Flavanoids and Glycosides are also responsible for the reduction of ulcerations in the colon [20 21].

Induction of colitis caused a significant reduction in the body weight and showed increase in the colon weight and spleen weight which is a good indicator of inflammation. EEAS administration causes significant reduction of spleen weight and colon weight and lesion area and improvement in the body weight of rats. The extract effectively reduced the histological signs of inflammation such as leukocyte infiltration, edema, and tissue injury.

Malondialdehyde is an endogenous product of enzymatic reactions that indicates signs of lipid peroxidation. Lipoperoxidation-derived aldehydes, like malondialdehyde (MDA) can damage proteins by generating covalent adducts whose accumulation probably participates in tissue damage.

Myeloperoxidase (MPO) is an enzyme that is found in neutrophils and monocytes/macrophages MPO catalyzes the formation of powerful reactive intermediates (hypochlorous acid [HOCl], tyrosyl radical, and reactive nitrogen intermediates) that can have profound biologic effects that result from modification of lipids and/or proteins. Intracellularly, MPO plays a major role in microbial killing but is released extracellularly at inflammatory sites after phagocyte activation and induces damage to host tissues. MPO

and/or its products have been observed in diseased tissues, including atherosclerotic lesions, synovial fluid of arthritic joints, and brain of patients with Alzheimer's disease. It is a good marker for the tissue injury, inflammation and neutrophil infilteration. Histopathological, biochemical, morphological parameters have showed evidence of decrease in pro-inflammation and increase in tissue healing. It is possible that along with anti inflammatory activity extract of *Allophylus serratus* helps in tissue regeneration which might contribute to its use in combating ulcerative colitis. In induction model MPO levels got increased, pre-treatment with ethanolic extract of *Allophylus serratus* showed significant reduction in the MPO levels.

Rats induced with DNBS showed decreased levels of glutathione (GSH), an antioxidant. Significant increase in the levels in rats was observed which are pre-treated with the extract.

The exact mechanism of induction is not clear but according to the previous findings it has been known that DNBS causes an oxidative damage which is accompanied by release of Reactive nitrogen species such as Nitric oxide, Peroxynitrite and Hydroxyl radicals which is a representation for tissue damage. DNBS induced rats showed increased levels of the NO where as rats pre-treated with the drug showed significant reduction in the NO levels. The augmentation of the endogenous antioxidant and reduction in oxidative stress will further boost the self-repair system of the body might also help against inflammatory damage.

CONCLUSION

From the present study it was concluded that the Ethanolic extract of *Allophylus serratus* showed significant reduction of colitis in rats, induced by DNBS in dose dependent manner. This effect might be due to presence of steroids, flavanoids, glycosides which are useful for reducing the ulcerations in colon by decreasing the levels of certain biochemical parameters and by improving the levels of antioxidants. Further research in isolation and identification of the phytoconstituent responsible for the anti colitis activity is needed to know the exact mechanism of action by which *Allophylus serratus* exhibits its therapeutic activity.

REFERENCES

1. Abhar EL.HS,Hammad.N.A, 2008,"Modulating effect of ginger extract on rats with ulcerative colitis". *Journal of Ethnopharmacology*; 118:367-372
2. Fiocchi.C,1998,"Inflammatorybowel disease; etiology pathogenesis.gastroenterology" 115:182-205
3. Sartor, R.B., 1997, "Pathogenesis and immune mechanisms of chronic inflammatory bowel disease". The American Journal of Gastroenterology 92, 5S–11S.
4. Dionne, S, 1997 "Quantitative PCR analysis of TNF-alpha and IL-1 beta mRNA levels in pediatric IBD mucosal biopsies". Digestive Diseases and Sciences, 42, 1557–1566.

5. Jainu, "Protective effect of *Cissus quadrangularis* on neutrophil mediated tissue injury induced by aspirin in rats". Journal of Ethnopharmacology 2006,104, 302-305
6. Munakata, 2003, Ecabet sodium attenuates reactive oxygen species produced by neutrophils after priming with bacterial lipopolysaccharides. Luminescence 18, 330-333
7. Cetinkaya, A, 2006, "Effects of L-Carnitine on oxidant/anti-oxidant status in acetic acid-induced colitis," Digestive diseases and sciences 51,488-494.
8. Nugent, A., 1979, "Levels of glutathione, glutathione reductase, glutathione-S-transferase activities in rat liver". Biochim.Biophys.Acta 582,67-68
9. Acharya, 2008, Indigenous Herbal Medicines: Tribal Formulations and Traditional Herbal Practices, Aavishkar Publishers Distributor, Jaipur- India
10. Manmeet kumar**,** 2010,"Anti-osteoporotic constituents from Indian medicinal plants",17(3):993-999
11. Cheng Bostwick, "Essentials of anatomic pathology",2006, 2nd edition, 1325-1327
12. Joshi SV, 2012," Protective effect of aqueous extract of *Oroxylum indicum* Linn. (Root bark) aganist DNBS-induced colitis in rats. *Indian journal of pharmacology"*, 43(6):656-660
13. Khan HA., 2004," Computer-assisted visualization and quantitaion of experimenatal gastric lesions in rats". Journal of pharmacol and Toxicol methods , 49:89-95
14. E.Sanmuga priya ,2012, "Phytochemical investigation of *Allophylus serratus* Kurz leaves by UV and GC-MS analysis", Reasearch journal of Phytochemistry 6(1):17-24
15. Krawisz, 1984. "Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity". Gastroenterology 87, 1344–1350
16. Eu jin cho, 2011, "Anti-inflammatory effects of methanol extract of *Patrinia - scabiosaefolia* in mice with ulcerative colitis", Journal of Ethnopharmacology,136,428-435
17. Ohkawa, H., 1979." Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction". Analytical Biochemistry 95, 351–358.
18. Owens, 1965." A colorimetric micro method for determination of glutathione," Biochemical Journal 94, 705–711.
19. Rastogi RP, Mehrotra BN,1990 " compendium of indian medicinal plants ", vol 4, new delhi: publication and information directorate; 85-86.
20. Rastogi RP, Mehrotra BN,1990 " compendium of indian medicinal plants ", vol 1, new delhi: publication and information directorate; 103-104.
21. Rastogi RP, Mehrotra BN,1990 " compendium of indian medicinal plants ", vol 3, new delhi: publication and information directorate; 92-94.