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EFFECT OF ALOE VERA LEAF EXTRACT IN ANIMAL MODEL OF ULCERATIVE COLITIS

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ABSTRACT

The present study was undertaken to evaluate the protective effect of aqueous leaf extract of *Aloe vera (ALEAV)* in animal model (wistar albino rats) through macroscopic and histopathological study of the colon after inducing ulcerative colitis by usage of acetic acid. 30 healthy albino rats of either sex 150-200g body wt. and 2-4 months age groups were divided into five groups (six in each). Group 1 was normal control group receiving vehicle 1ml of normal saline transrectally (negative control). Group 2 was ulcerative colitis control which was treated with normal saline. Group 3 & 4 were administered aqueous leaf extract of *Aloe vera* suspension(test drug) 150,300mg/kg bodyweight dose orally for 10 days respectively. Ulcerative colitis was induced on 10th day. Group 5 was administered prednisolone(1mg/kg) orally for 3 days. On the 3rd day ulcerative colitis was induced by transrectal administration of 1 ml of 4% acetic acid solution. In all the groups, animals were observed for decrease in bodyweight, stool consistency and rectal bleeding i.e Disease Activity Index for 48 hours after inducing ulcerative colitis. After 48 hours the rats were sacrificed by high dose of ether anaesthesia and a piece of colonic segment(10cm in length) were resected out and subjected to macroscopical study(colon mucosal index) and microscopic evaluation. ALEAV at 150 mg/kg produced significant improvement in macroscopic score in comparison to disease control (group 2) which is comparable to that of normal control (group 1) in ulcerative colitis model. Histopathological studies showed significant dose dependent decrease in lymphoid hyperplasia, neutrophilic infiltration, crypt damage and submucosal inflammation in ALEAV treated group (150 and 300 mg/kg b.w) in comparison to disease control.

KEYWORDS: aloevera leaf extract, ulcerative colitis, disease activity index, colon mucosal index.

INTRODUCTION

Ulcerative colitis is a chronic inflammatory bowel disease. It can affect equally both males and females. It is a familial disease. The disease has got potential risk towards development of colon cancer.[1] Till now the therapeutic options are very limited and the results are not so encouraging. A meta-analysis showed a significant increase in risk for lymphoma in ulcerative colitis patients treated with azathioprine or 6-mercaptopurine (immunosuppressant). Mesalamine and sulfasalazine are generally considered to be the mainstay in the treatment of ulcerative colitis but a major group of patients (17%) experience non-bloody diarrhoea according to studies.[2] Corticosteroids are ineffective in sustaining the medically induced remission.[3] Other immunosuppressant like cyclosporine, tacrolimus and methotrexate produce effective results but toxicities of these groups of drugs limits their use. Biologics though giving the patients very good quality of life but the affordability arrests their use to common people. Population based studies suggest, despite correct administration of conventional therapy in ulcerative colitis patients, they develop severe complications. So also the risk/benefit ratio is very high of the available conventional medications used in ulcerative colitis. Hence search is going on to get a better therapeutic option to halt and control the disease and to provide the patients of ulcerative colitis a good quality of life.

Aloe vera is an herb that has frequently been used in natural remedies. It is believed to have positive effects on skin and wound healing. Literature revealed *Aloe vera* extract exhibited potent anti-inflammatory activity in both chronic and acute model of inflammation.[4] Hypoglycaemic and ulcer protective effects of *Aloe vera* extract are clearly explained in various research articles, in experimental albino rats.[5] But, diverse results have been documented regarding use of *Aloe vera* in inflammatory bowel disease. Hence, availability of variable results and scanty data prompted us to evaluate the effect of *Aloe vera* extract in animal model of ulcerative colitis.

AIM AND OBJECTIVES

The present study was planned and designed with the following objectives:

- ❖ To evaluate the protective effect of aqueous *Aloe vera* extract in animal model (wistar albino rats) of ulcerative colitis.
- ❖ To perform a histopathologic and macroscopic study after induction of ulcerative colitis to assess the protective effect of aqueous *Aloe vera* extract.

MATERIALS AND METHODS

This prospective animal study was carried out in Department of Pharmacology, Srirama Chandra Bhanja Medical College And Hospital, Cuttack during the period from May 2013 to July 2013, after approval of Institutional Animal Ethics Committee(IAEC) on17.08.12.

1. Preparation of aqueous leaf extract of *Aloe vera*

Full size mature leaves were cut from the plant and rind removed. To make the gel thicker, the plant's leaves were given sufficient time to be relatively dried at room temperature without exposure to direct sunshine. After adding small quantity of water the leaves were ground in a blender and centrifuged at 10000g to remove the fibres and filtered through filter papers. The filtered aqueous extract was stored at 4^0C before use and allowed to warm up to room temperature a few hours before.

2. Acetic Acid 4%(pH=2.4)
3. Phosphate Buffer Solution(pH=7)
4. Formalin 4%

Animals

Thirty(30) healthy albino rats of either sex 150-200gm body weight, placed in polypropylene cages in a well ventilated room of our central animal house were used for the study. They were allowed free access to rat feeds and clean tap water except while fasting was going on. Rats were deprived of food for 12h prior to induction of ulcerative colitis but were allowed free access to tap water throughout experiments.

METHOD

1. Induction Of Ulcerative Colitis

Ulcerative colitis was induced by transrectal administration of 1ml of 4% acetic acid solution(pH=2.4) under low dose of ether anaesthesia after overnight fasting. The rat was maintained in head down position for 30 seconds to prevent leakage. The rest of the solution was aspirated out after which 2ml of phosphate buffer solution with(pH=7)was administered transrectally.

2. Study Design

The study comprised of five groups of six animals each as follows.

- Group 1: normal control-receiving vehicle 1ml of normal saline transrectally (negative control)
- Group 2: ulcerative colitis control-treated with normal saline
- Group 3 & 4: aqueous extract of *Aloe vera* suspension(test drug) 150,300mg/kg bodyweight dose was administered orally for 10 days respectively. Ulcerative colitis was induced on 10^{th} day.
- Group 5: prednisolone(1mg/kg) was administered orally for 3 days. On the 3^{rd} day ulcerative colitis was induced. In all the groups, animals were observed for decrease in bodyweight, stool consistency and rectal bleeding i.e Disease Activity Index for 48 hours after inducing ulcerative colitis. After 48 hours the rats in all the groups were sacrificed by high dose of ether anaesthesia and a piece of colonic segment(10cm in length) were resected out and subjected to macroscopical study(colon mucosal index) and presented with 4% formalin for microscopic evaluation.

Parameters evaluated

1. Disease Activity Index(DAI)

The DAI during study period(48 hours) was scored as follows:

0-no weight loss, normal stool consistency, no rectal bleeding

1 = weight loss(1-5%), normal stool consistency, no rectal bleeding

2 = weight loss(5-10%), loose stool, no rectal bleeding

3 = weight loss(10-20%), normal stool consistency, no rectal bleeding

4 = weight loss(>20%), diarrhoes, gross rectal bleeding

2. Colon Mucosal Index

Macroscopic Score

After resection of portion of colon, it was opened by midline incision and rinsed with saline too and was observed from lumina side with the help of a magnifying glass.

Scoring pattern was as follows:

0=normal mucosa

1=mild hyperaemia and edema

2=moderate hyperaemia, edema and erosion

3=severe hyperaemia, edema, necrosis, ulcer less than 1cm

4=severe hyperaemia, edema, erosion and ulcer of more than 1cm

Microscopic Score

The histopathology score was assessed as follows:

1=infiltration of inflammatory cells

2=deposition of fibrin protein

3=submucosal neutrophil migration

4=submucosal edema

5=epithelial edema

Statistical analysis

The data were analysed by one way ANOVA followed by tukey's multiple range test. $P<0.05$ will be considered significant. Graph pad prism software was used for this purpose.

RESULTS

Table-1 Effect of aqueous leaf extract of *Aloe vera* (ALEAV) on acetic acid induced ulcerative colitis

Groups	Disease activity index (DAI)	Colon mucosal index(Macroscopic score)	Colon mucosal index (microscopic score)
Control (group 1)	0 ± 0	0 ± 0	0 ± 0
Model (group 2)	7.72 ± 0.47	3.02 ± 0.27	3.74 ± 0.25
Prednisolone (group 5)	0 ± 0	0 ± 0	0.24 ± 0.25
AEAV (150 mg/kg) (group 3)	3.68 ± 1.41	0.73 ± 0.25	0.51 ± 0.28
AEAv(300 mg/kg) (group 4)	0.02 ± 0.01	0.22 ± 0.20	0.25 ± 0.25
P	P < 0.0001	P < 0.0001	P < 0.0001

*Values expressed as mean ± SEM, n= 6 in each group, *P < 0.01 when compared with control,**P < 0.0001 when compared with model*

Table-1 showed effect of aqueous leaf extract of *Aloe vera* (test drug) and prednisolone (standard drug) on ulcerative colitis model in albino rats. Parameters studied were DAI and CMI (includes both macroscopical and microscopical findings). disease activity index was assessed by changes in body weight, stool consistency and rectal bleeding during 48 hours period following colitis induction. The groups (3,4) treated with AEAV at doses of 150mg/kg and 300 mg/kg produce significant improvement in Disease Activity Index in comparison to disease control model. There was no significant difference between the two doses of ALEAV in the model.

The parameters used for evaluating the degree of inflammation in both the models were change in mucosal pattern and severity of lesions. ALEAV at 150 mg/kg produced significant improvement in macroscopic score in comparison to disease control (group 2) which is comparable to that of normal control (group 1) in ulcerative colitis model (fig 1 and -2).

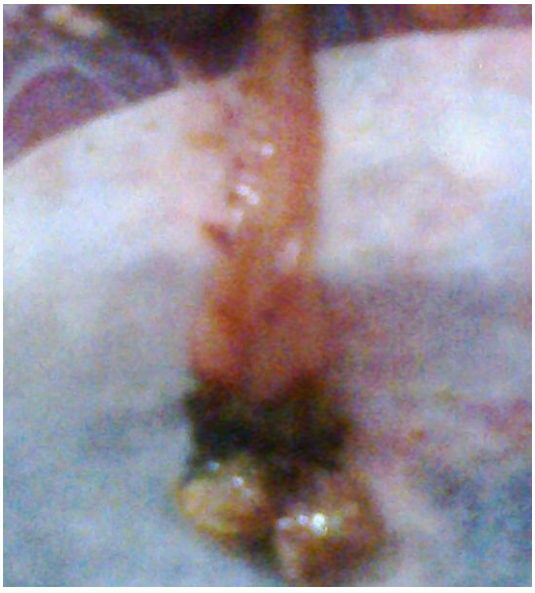

Fig-1

Fig-2

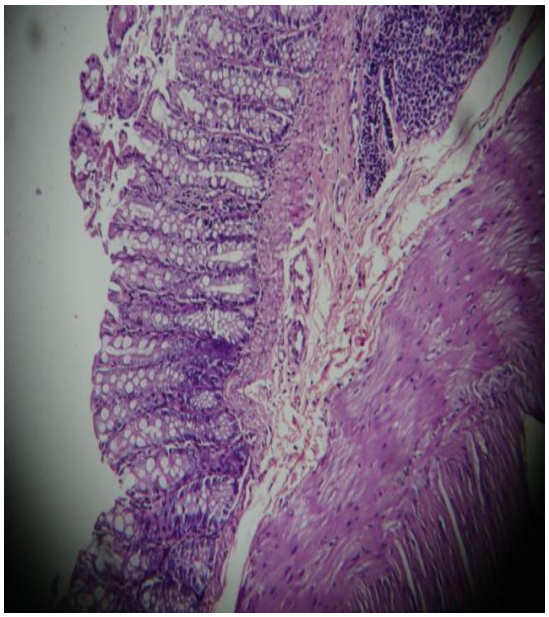

Fig-3

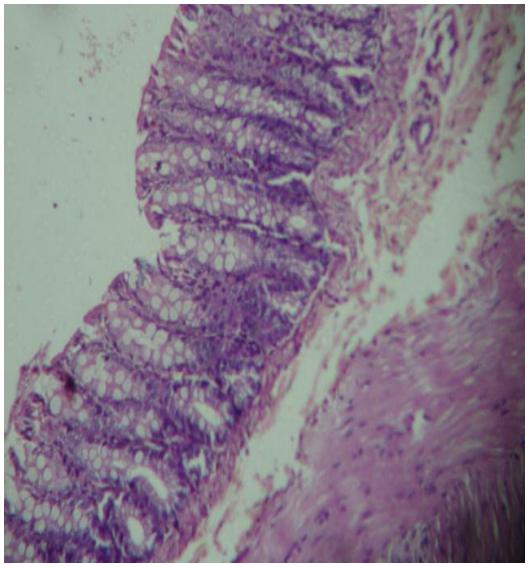

Fig-4

Histopathological analysis of colon in acetic acid induced ulcerative colitis clearly showed that there was significant degree of lymphoid hyperplasia, neutrophilic infiltration, crypt damage and mucosal inflammation compared to normal control. But there was significant dose dependent decrease in lymphoid hyperplasia, neutrophilic infiltration, crypt damage and submucosal inflammation in AEAV trated group (150 and 300 mg/kg b.w) (fig no. 3 and 4).

DISCUSSION

Ulcerative Colitis and Crohn's Disease are the common inflammatory bowel disease seen in day to day clinical practice. A study to see the protective effect of aqueous leaf extract of *Aloe vera* on an easily inducible ulcerative colitis model which produces mucosal injury. Acetic acid induced inflammation and generation of reactive oxygen species is the standard procedure for induction of ulcerative colitis.

The model shares many histopathological and clinical features of human ulcerative colitis useful for the study of the protective effect of AEAV on chronic inflammation as well as providing an inexpensive model suitable for assessing new therapeutic agents.[6]

In the present study, ulcerative colitis was induced by intra-rectal administration of acetic acid in animals. The severity of colonic inflammation in developed disease was evaluated by measuring main parameters DAI and CMI score respectively. Reduction of body weight is a sign of generation of ulcerative colitis which is later confirmed by microscopic and macroscopic findings.

Effect of aqueous leaf extract of *Aloe vera* was observed on the above experimental animal model of ulcerative colitis in albino rats. The extract was administered continuously for ten (10) days and on the 11th day of the study period, ulcerative colitis was induced by acetic acid. The animals were observed for 48 hrs and then, sacrificed by high dose ether anaesthesia. Body weight, stool consistency, bloody diarrhoea, macroscopic and microscopic findings were constantly recorded.

The results showed a significant decrease in severity of colonic inflammation following treatment with *Aloe vera* (both in 150 and 300 mg/kg body wt. doses). prednisolone was used as a standard drug which produced no significant difference in the above parameters in comparison to ALEAV at all doses.

Aloe vera traditionally known as **"ghrita kumari"**, is a perennial plant having thick fleshy leaves from which a thick juice flows when it is transversely cut.[7] Multiple ingredient have been found in Aloe leaf like lignin, saponin, anthroquinones, minerals, vitamins, amino acids, enzymes, sugars and sterols.[8] The biological activity of *Aloe vera* gel is suggested to be synergistic action between the polysaccharide base and other components. Mannose-6-phospate, a major polysaccharide in *Aloe vera* leaf has a role in wound healing and anti-inflammatory activity.[9] The leaf gel polysaccharides, especially the acetylated mannaose have been seen to possess immunomodulatory properties.[10] A study done by Sabeh, Farideh (1995)[11] in the University of North Texas found two antioxidant enzymes, glutathione peroxidase and superoxide dismutase (SOD) involved in scavenging oxygen species while purifying the *Aloevera* plant by ionexchange chromatography. They also found that the aloevera SODs have high specific activitiesthese high activities may relate to plant's healing properties of inflammatory disorders in ulcerative colitis. As dysregulated immunity is the major etiological factor of ulcerative colitis and scientific evidences suggest *Aloe vera* possesses immunomodolatory activity tested in murine and human lymphoid cells, so it can be very well tried in the management of ulcerative colitis. Dr Bland's study aimed to "evaluate the effect of oral *Aloe vera* juice supplementation of gastric pH, stool specific gravity protein digestion/absorption and stool microbiology" and found that it could be used in the treatment of inflammatory bowel disease.[5]

CONCLUSION

This study revealed that the aqueous extract of *Aloe vera* provides protection on induced inflammation in ulcerative colitis. The protective effect is comparable to the protection offered by prednisolone in animal model of ulcerative colitis.

But this study could not explain the possible mechanism of protective effect of *Aloe vera* and its link to prednisolone. A more in depth study is required with a larger numbers and controls to further investigate the changes seen and look into the mechanisms pertaining to the protective effect of *Aloe vera*.

The potential benefits of *Aloe vera* and mechanisms underlying if unraveled through further research can surely revolutionize the treatment of ulcerative colitis.

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