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Study on the effect of Amlodipine on chemically induced inflammatory bowel disease using animal model.

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Abstract: The objective of this study is to investigate the effect of Amlodipine, a L- type calcium channel blocker on chemically induced ulcerative colitis in mice based on the hypothesis of blocking calcium channels in the colon leads to decreased the levels of neuropeptides which is responsible for ulcerative colitis. 0.1 ml of (6% v/v) acetic acid (AA) was used to induce ulcerative colitis. After seven days treatment with Amlodipine blood was collected from retro orbital puncture, serum was separated and total protein (TP) and total hemoglobin (Hb) were measured. Colon was excised, washed and its histological changes and spleen weight were also measured. Colon tissue homogenate was subjected to measure glutathione content and nitric oxide production (NO). Intracolonic administration of Amlodipine significantly reduced the severity of AA induced ulcerative colitis. Serum concentrations of TP, Hb levels also increased into normal levels. Amlodipine prevents spleen enlargement, glutathione depletion and nitric oxide production effectively in inflammed colonic tissue. The present study concluded that the Amlodipine, a calcium channel blocker possess significant reduction in inflammation against acetic acid induced ulcerative colitis in mice.
Key words: Inflammatory bowel disease, Amlodipine, Acetic acid, Ulcerative colitis, Neuropeptides.

Introduction

Ulcerative colitis (UC) and crohn's disease (CD) are chronic inflammatory diseases generally called as inflammatory bowel disease which occurs in gastrointestinal tract. The molecular biological mechanisms and pathogenesis are not yet known[1]. UC affects only the large intestine by pathological ulceration and mucosal damage; CD which affects any part of the gastro intestinal systems characterized by chronic and spontaneously relapsing inflammation[2]. Diarrhea, weight loss, abdominal pain and nausea are the symptoms of IBD. Genetic, immunologic and environmental factors which may involved in the etiology of IBD[3]. Currently using drugs for IBD having lot of side effects; 5 –amino salicylic acid derivative drugs is associated with diarrhea, cramps and abdominal pain. Corticosteroids produce immunosuppression, rounding of face, high blood pressure, acne, diabetes, weight gain, increased body hair. Immunomodulator agents, antibiotics and Anti TNF-α also having many disadvantages. We need drugs which effectively decrease the colonic inflammation with fewer side effects[4]. A number of animal models have been developed to explore the etiology and therapeutic method for IBD. Acetic acid induced colitis is one of the animal models that resembled to human ulcerative colitis in based on the acute inflammatory responses[2]. The enteric nervous system has turned out into new pharmacological options for the treatment of gastro-intestinal diseases called as neuro- gastrointestinal pharmacology[5]. The normal functions of enteric neurons including motility, blood flow, mucosal growth secretion and local immune system. During the course of IBD a transient structural alterations takes place in the ENS[6]. Any peptides released from enteric neurons called as neuropeptides and plays an important role in IBD. Some of the neuropeptides neurotensin, vasoactive intestinal peptide, gallanin, substance P increase the severity of IBD[7]. Neuropeptides blockade is the new therapeutic approach for IBD. These peptides synthesized, stored in large dense core vesicles and released in the synaptic cleft in related to calcium dependent depolarization[8]. We hypothesized that calcium channels blockade in the large intestines and colon will be diminish the neuropeptides levels responsible for the inflammatory bowel disease. Among the four types of calcium

channels; L type calcium channels are most abundantly present in intestines and colon. So we selected to investigate an L type calcium channel blocker amlodipine for this study.

Animals

Swiss albino female mice (20-30g; n=6 per group) were purchased from National Centre for Laboratory Animal Sciences at National Institute of Nutrition, Hyderabad. They were maintained under standard laboratory conditions and provided with standard diet (Amruc food) and water *ad libitum*. The experimental protocol has been approved by Institutional Animal Ethics Committee Rajah Muthaiah Medical College, Annamalai University, Reg No.160/1999/CPCSEA, Proposal number – 1009.

Induction of experimental ulcerative colitis

Mice were fasted for twenty four hours and slightly anesthetized with ketamine injection (24 mg/kg IM)[9]. To induce colitis 0.1 ml of acetic acid solution (AA) (6% v/v)[10] is diluted with distilled water was instilled into the lumen of colon by using a rubber catheter and the tip was 4cm proximal to the anus, then the mice were maintained in a supine trendelenburg position for 30 seconds is to prevent the leakage of intracolonic instill[11].

Experimental Design

Animals were divided into five groups, each consisting of minimum six in a group

Group-1 Control animals received vehicle.
Group-2 Colitis animal received vehicle.
Group -3 Colitis animal received AML (0.65mg/kg).
Group -4 Colitis animal received AML (1.3mg/kg).
Group -5 Colitis animal received Prednisolone (5mg/kg)[12]

AML doses was fixed by calculating from human doses by using a standard formula[9] and made a suspension by using water and polyethylene glycol. All treatments were administered per oral (p.o) and continued up to seven days, on the 8th day the animals were anaesthetized with ketamine (24mg/kg) and blood was collected by retro orbital puncture, for biochemical estimation serum was separated and stored under at -80° C. The animals were euthanized by cervical dislocation and colonic segments were excised, washed with cold saline and were used to measure colonic weight and histopathological examination.

Serum Estimation

The total proteins (TP), total haemoglobin (Hb) were estimated as per the standard procedure given in the kit using a semi- auto analyzer (Humalyzer 3000).

Biochemical Estimation

The colon tissues were weighed, homogenized with Phosphate buffer (pH 7.0) was used to measure reduced glutathione (GSH) and nitric oxide (NO)[13].

Estimation of reduced glutathione (GSH)

Homogenate (0.5ml) was precipitated with 2 ml of 5% TCA, Ellman's reagent (0.5ml) and 3 ml of phosphate buffer (pH 8.0) was added and the mixture was centrifuged at 3200 ×g for 20 min. The absorbance was read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3.5 ml of buffer. The values were expressed as mg/100 g-tissue[14].

Assessment of Nitric oxide (NO)

The nitrite concentration was measured in the supernatants of tissue homogenate with 1% bovine serum albumin. Equal volume of the sample is mixed with griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% N-[1- Napthyl]-ethylenediamine) were mixed and measured the absorbance at 450 nm. The amount of nitrite was obtained by an extrapolation from a standard curve with sodium nitrite and was expressed as µmol/mg tissue[15].

Histopathological Study

Part of distal colon of different groups of mice was fixed immediately in 10% formaldehyde solution, embedded in paraffin, cut into 5mm thick transversal sections, mounted on glass slides, deparaffinized and stained with hematoxylin and eosin stain (HE). The images were obtained using a light microscope[15].

Statistical Analysis

All data expressed as mean ± SEM statistical significance was measured by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test using Graphpad prism version 5.0 and values of $p<0.05$ were considered as statistically significant.

Results and Discussions

Spleen weight is a reproducible marker of systemic inflammation (Fig.1) raised significantly in AA induced colitis group when compared with normal control group. AML (0.65mg/kg) produce insignificantly reduced spleen weight compared with AA induced colitis control group. AML (1.3mg/kg) and Prednisolone (5mg/kg) produce significant results when compared with AA induced colitis group.

TP and total Hb were decreased significantly ($p<0.001$) in AA induced colitis group compared with normal control group. AML (0.65mg/kg) produce insignificantly (ns) increased TP levels compared with AA induced colitis group. In contrast AML (1.3mg/kg) significantly ($p<0.01$) increased TP levels levels compared with AA induced colitis group. During chronic inflammation, anorexia or illness, oxidative injury will lead to decrease serum total protein levels. In ulcerative colitis patient's blood loss occurs with faeces, so hemoglobin level was generally decreased

AML (0.65mg/kg) produce significantly ($p<0.05$) increased Hb levels compared with AA induced colitis group. AML (1.3mg/kg) significantly ($p<0.001$) increased Hb levels levels compared with AA induced colitis group. Prednisolone (5mg/kg) produce significant ($p<0.001$) increased in both TP and Hb levels when compared with AA induced colitis group.

Colonic GSH levels (**Fig.4.**) are significantly ($p<0.001$) reduced in AA induced colitis group compared with normal control group. AML (0.65mg/kg) produced significant ($p<0.01$) results compared with AA induced colitis control group. AML (1.3mg/kg), Prednisolone (5 mg/kg) significantly prevents depletion of glutathione content in the colon.

Multiple antioxidant systems like antioxidant enzymes and low molecular weight antioxidants (Reduced glutathione) are present in the colonic epithelium. GSH is mainly act as a cytoprotective and it protects gastric mucosa. In ulcerative colitis patient's depletion of tissue GSH leads to marked cellular degeneration of the colon epithelium.

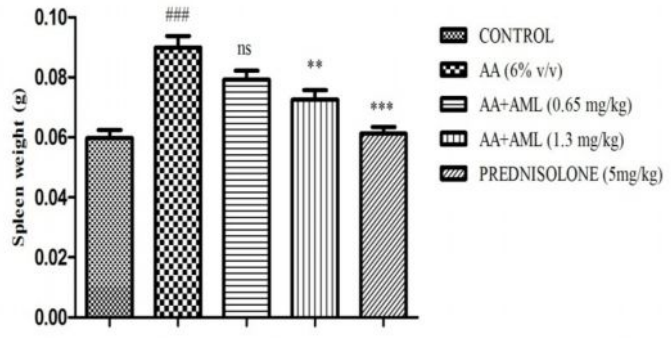


Fig. 1.Change in spleen weights (g)

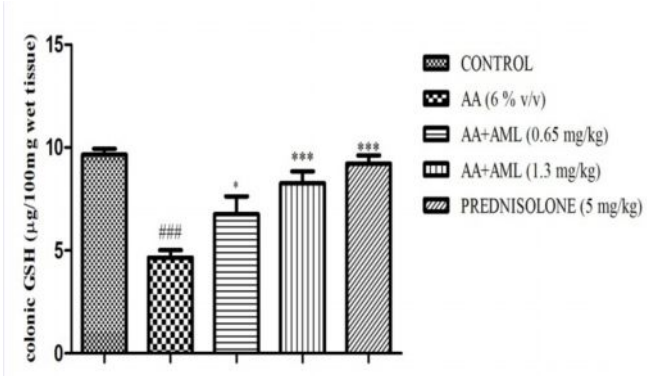


Fig.2.Total protein levels (gm/dl)

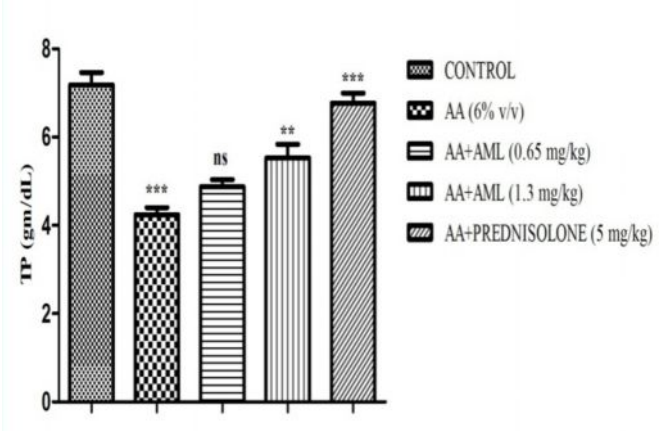


Fig.3. Hb levels (gm/dl)

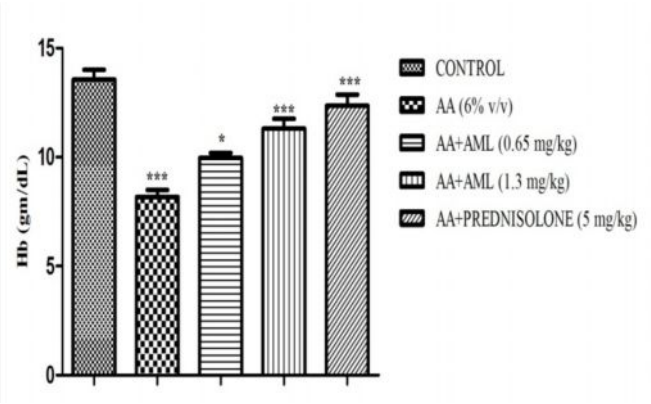


Fig.4.Colonic GSH level (µg/100g wet tissue)

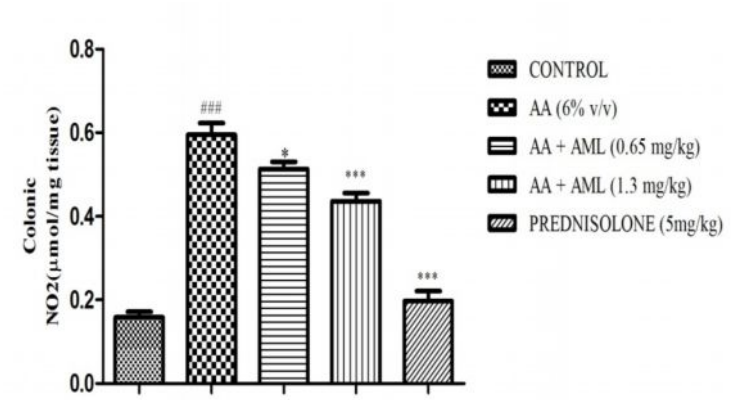
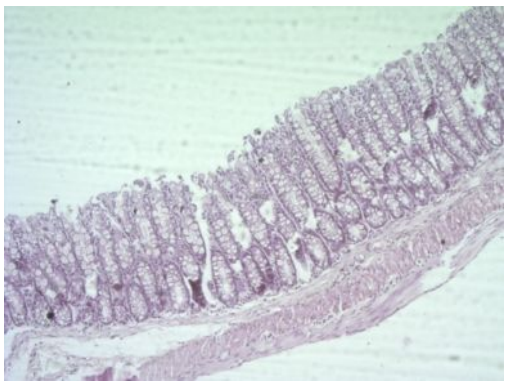
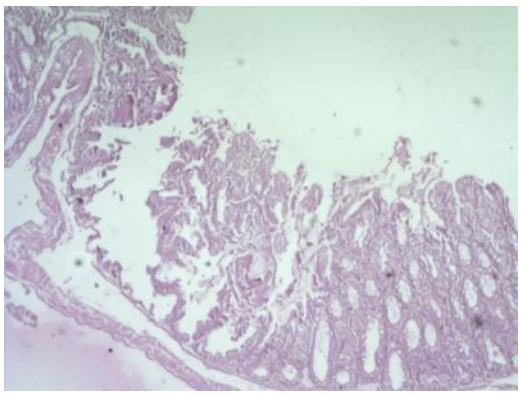


Fig.5.Colonic NO (µmol/mg tissue)

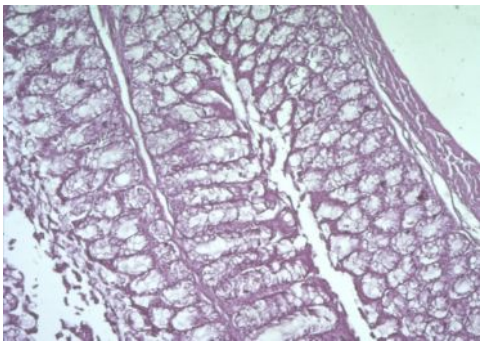
Colonic nitric oxide levels are significantly ($p<0.001$) increased in AA induced colitis control group compared with normal control group. AML (0.65mg/kg) shows less significant results ($p<0.05$) on colonic nitric oxide levels when compared with colitis control group. AML (1.3mg/kg) and Prednisolone (5mg/kg) significantly ($p<0.001$) prevents nitric oxide formation compared with AA induced colitis control group and results were shown in **Fig.5**. Nitric oxide overproduction via induced nitric oxide synthase upregulation by colonic epithelium is related to inflammatory bowel disease particularly ulcerative colitis. AML at the both doses significantly reduced nitric oxide production.



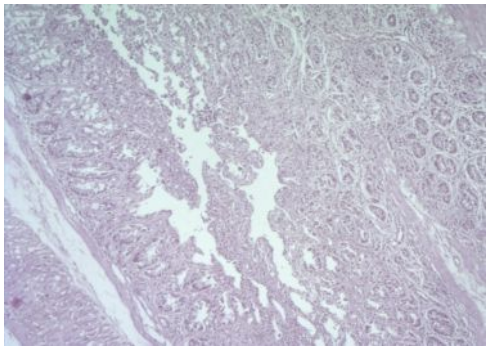
[A] Control group



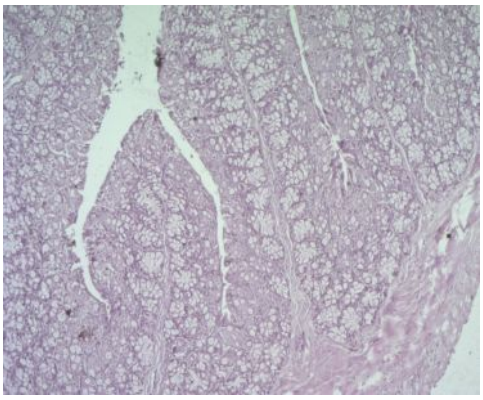
[B] AA (6%) induced colitis mice colon



[C] AA+ AML (0.65 mg/kg) treated mice colon



[D] AA+ AML (1.3 mg/kg) treated colon



[E] AA+PREDNISOLONE (5 mg/kg) treated colon.

Fig.6. Effect of AML on histological analysis (H&E×100x) stained sections of colon.

In control groups the histological results showed no damages in epithelium, mucosa and submucosa. In AA induced colitis colon showed loss of surface epithelium, damages in crypts and submucosa. AML (0.65mg/kg) treated colon shows mild regeneration of surface epithelium and crypts. AML (1.3 mg/kg) treated colon showed prominent fibroplasias with regeneration of epithelium and crypts. From the Histological results AML attenuates the severity of acetic acid induced colitis and helps to regeneration of tissues.

Conclusion:

The possible mechanism may be inhibiting the neuropeptides which is responsible for the severity of ulcerative colitis. The present study concluded that the Amlodipine, a calcium channel blocker possess significant reduction in inflammation against acetic acid induced ulcerative colitis in mice.

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