

Research Article

Role of Chinnodbhavadi Kwath- An Ayurvedic Formulation on the Experimental Colitis Induced By Acetic Acid in Rats

Mukeshkumar Nariya¹, Vinay Shukla², Basavaiah Ravishankar³ and Sunita Jain^{4*}

¹Department of Pharmacology, Ayurveda Contraceptive Drug Research Institute (CCRAS), Ahmedabad, Gujarat, India

²Chemistry Laboratory, Institute of Postgraduate Teaching and Research, Gujarat Ayurved University, Jamnagar, India

³SDM College of Ayurveda, Kuthpadi, Udupi, India

⁴Department of Pharmacology, L.M. College of Pharmacy, Gujarat University, Ahmedabad, Gujarat, India.

ABSTRACT

Triphala formulation is one of the renowned formulation used alone or along with other ingredients in Ayurvedic therapeutics for the treatment of gastro-intestinal problems including large intestinal inflammation and colitis. The purpose of the present study is to evaluate the role of one of the Triphala formulation called as Chinnodbhavadi kwath (decoction) in modulating the extent and severity of experimental ulcerative colitis in rats. Colitis was induced by intra-colonic instillation of 2 ml of 4% (v/v) acetic acid in rats. The inflammatory response was assessed by effect on colonic fluid transport, macroscopic scoring and tissue biochemical parameters. In acetic acid colitis, the net colonic fluid absorption became secretory and this degree of alteration was reversed at some extent by Chinnodbhavadi kwath. The test drug attenuated the inflamed colonic myeloperoxidase activity and showed increased in glutathione and superoxide dismutase content. These findings demonstrate the moderate effect of Chinnodbhavadi kwath in down regulation of inflammatory response in acetic acid-induced colitis in rats possibly by its antioxidant properties.

Keywords: Chinnodbhavadi kwath; Triphala; Colonic fluid absorption; Antioxidant property.

INTRODUCTION

Inflammatory bowel diseases (IBD) that includes ulcerative colitis and crohn's disease are common and chronic gastro-intestinal disorders characterized by intestinal inflammation and mucosal tissue damage. The etiology of IBD remains unknown, although it is believed that deregulated immune response along with several intra and extra multi-factorial intestinal manifestations including environmental, genetic and auto immune phenomena influences both the initiation and progression of IBD¹. In fact, efficient drugs currently used in the treatment of IBD, such as 5-aminosalicylic derivatives, are not devoid of potentially serious side effects, especially when used at high doses or during prolonged period of time, thus limiting their use². This throws up the possibility of using plant based products,

which may not have serious side effects, to treat these conditions³.

Triphala formulation is one of the renowned formulation used alone or along with other ingredients in Ayurvedic therapeutics for the treatment of gastro-intestinal problems. It is categorized as rejuvenator⁴ and reported to be an antioxidant rich herbal formulation⁵. Triphala is traditionally been used as laxative, colon cleansing, digestion problems and poor food assimilation. It is also used in intestinal inflammation and ulcerative colitis⁶. As per Ancient text, one of the Triphala formulation called as Chinnodbhavadi kwath (decoction) is used for chronic hyperacidity and gastro-intestinal problems⁷. Though, wide usage of Triphala formulation called as Chinnodbhavadi kwath in gastrointestinal disorders in practice, to date, the possible

modulatory role of Chinnodbhavadi kwath in colon inflammation in animals has not been yet verified; hence we aimed in the current investigation to evaluate the possible modulating effect(s) of Chinnodbhavadi kwath on acetic acid-induced ulcerative colitis model in rats.

EXPERIMENTAL

Plant drugs and chemicals

Stem of *Tinospora cordifolia* (Willd.) Miers. (Menispermaceae), stem bark of *Azadirachta indica* A. Juss. (Meliaceae) and leaves of *Trichosanthes dioica* Roxb. (Cucurbitaceae) collected from forest of Barda hills, Jamnagar (Gujarat, India) in the month of September and October (2005). Fruits of *Terminalia chebula* Retz. (Combretaceae), *Terminalia belerica* (Gaertn) Roxb. (Combretaceae) and *Embllica officinalis* Gaertn. (Euphorbiaceae) collected from forest of Dang and Valsad (Gujarat, India) in the month of December (2005) were used in the study. The plant materials were authenticated and voucher specimens of each submitted to Pharmacognosy laboratory of IPGT and RA, Gujarat Ayurved University, Jamnagar, India. Chinnodbhavadi kwath (decoction) was prepared by mixing equal proportion of *T. chebula*, *T. belerica*, *E. officinalis*, *T. cordifolia*, *A. indica* and *T. dioica*. Coarse powder (48 g) of mixture and 768 g water was added; boiled on low to medium heat till the liquid portion was reduced to 1/8th of the original volume (96 g) and filtered⁸. The test formulation and their ingredients were standardized using gallic acid as a marker compound by HPTLC finger Print. Gallic acid was observed at 0.52 Rf value, when scanned at 254 nm. The ingredients and formulation show the almost same Rf values as observed for gallic acid. The concentration of trace heavy metals such as lead, cadmium, arsenic and mercury present in formulation were analyzed by Atomic Absorption Spectrophotometer. As could be observed from the study, trace metals do not seem to be present in significant quantities in Chinnodbhavadi kwath. All chemicals used in the study and for biochemical assay were of analytical grade.

Animals

Inbred Wistar albino rats of either sex, weighing between 180 to 220 g, were used for the study. The animals were maintained under ideal husbandry conditions and reared under standard condition of temperature, humidity and exposed to 12 h light and dark cycles. All animals were exposed to the same environmental conditions and were maintained on standard diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee as per guideline of *Committee for the Purpose of Control and Supervision on Experiments on Animals*, India.

Acute toxicity study

Acute oral toxicity of Chinnodbhavadi kwath was carried out in female rats as per the 423 guideline of OECD (Organization of Economic Co-operation and Development)⁹. The result showed that Chinnodbhavadi kwath did not produce any changes in observed parameters and mortality at dose of 2000 mg/kg. Hence, animal dose of Chinnodbhavadi kwath were fixed on the basis of human therapeutic dose mentioned in literatures^{8,10}.

Induction of colitis

Rats were randomized in to four groups, each consisting of eight animals. Group (I) normal control group and Group (II) colitis control group, were received vehicle as an aqueous suspension of 1% carboxymethyl cellulose (CMC) in dose of 0.5 ml/100 g body weight. Group (III) was test drug treated groups which received Group (III) was drug treated groups which received Chinnodbhavadi kwath in a dose of 8.7 ml/kg body weight of rat. The drugs were suspended in 1% CMC and administered orally once daily for seven consecutive days. Group (IV) rats were treated with sulafasalazine 48 h, 24 h and 1 h prior to induction of colitis in a dose of 100 mg/kg and used as a positive control group¹¹. The animals were fasted for 24 h with access to water *ad libitum* before induction of colitis¹². On 7th day, 1 h after drug administration, fasted animals were slightly anaesthetized with anesthetic

ether. Under anesthesia an enema of 2 ml of 4% (v/v) acetic acid was infused in to lumen of colon by using polyethylene tube (2 mm in diameter) which was inserted through rectum in to the colon to a distance of 8 cm proximal to the anus verge. Rats from normal control group received 2 ml of phosphate buffer saline. After 24 h, the animals were studied for *in vivo* colonic fluid absorption and afterwards they were sacrificed. Colon was dissected out for assessment of colonic damage and biochemical studies.

***In vivo* colonic fluid absorption**

The tied off rat colon technique was used and modified for the experimental need¹³. The animals were anesthetized with urethane (1.2 g/kg, ip) and kept warm under lamp through out the experiment. After laprotomy, the colon was exposed and proximal occluding ligature was placed 2 cm distal from the ceco-colonic junction. The luminal contents were thoroughly flushed out from the colon with 20 + 20 ml of saline kept at 37°C. The lumen was then dried out by injecting 20 + 20 ml of air and further gentle manual expression. A second distal occluding ligature was then placed 1 cm above the rectal plaque. One ml of warm tyrode solution was carefully injected in to proximal part of colon using 27 gauze needle and the proximal ligature was closed. The colon was returned to the abdominal cavity and the incision was sutured and animal left for exactly one hour. At the end of period animal sacrificed and tied off colon carefully removed. The remaining fluid collected in the tube and weighed. A positive difference between the initial volume and fluid remaining (As determined gravimetrically) was considered as absorption and negative values considered as secretion. The results were expressed as $\mu\text{l/h.g}$ wet tissue.

Assessment of colonic damage

The colon was rapidly excised, cleaned of fat and mesentery and opened along its anti-mesenteric border, gently rinsed of its luminal contents with cold saline solution. The colon was scored for macroscopic visible damage on a 0 to 10 scale by

observer unaware of the treatment according to the criterium described by Bell et al. (1995)¹⁴ which accounts the extent of damage as well as severity of the colon damage.

Biochemical analysis

The colon was subsequently divided in to longitudinal pieces and stored immediately at -20°C for estimation of tissue biochemical parameters. Protein content was quantitated using bovine serum albumin as a standard and was expressed as mg/g wet tissue¹⁵. Myeloperoxidase activity as an index of the neutrophil infiltration in to the colonic mucosa was determined by previously described method¹⁶. One unit of myeloperoxidase activity is defined as that required to convert 1 μmol of hydrogen peroxide in to water per min at 37°C and was expressed in units per gram (Unit/g) of wet tissue. Superoxide dismutase (SOD) activity was determined by the nitro blue tetrazolium reduction method¹⁷. Lipid peroxidation (LPO) was measured as thiobarbituric acid reactive substances (TBRAS) formation as described previously¹⁸. TBRAS concentrations were calculated by the use of malondialdehyde (MDA) as a standard and results were expressed as nmol MDA/g tissue. Glutathione present was estimated and the results were expressed as nmol/g wet tissue¹⁹.

Statistical analysis

The data are expressed as mean \pm standard error of mean for eight rats per experimental group. One way analysis of variance (ANOVA) was used to compare the mean values of quantitative variables among the groups followed by Dunnet's multiple 't' test for unpaired data to determine significant difference between groups at $p < 0.05$.

RESULT AND DISCUSSION

Induction of colitis by acetic acid in rats is one of the standardized and well established commonly used methods to produce an experimental model of inflammatory bowel disease with some resemblance to human acute intestinal inflammation²⁰. The rectal administration of acetic acid produced severe

macroscopic mucosal inflammation, ulceration and hemorrhagic lesions in the colon of rats, as observed by significant increase ($p < 0.001$) in colonic damage score compared to normal control group of rats (Table 1). This parameter was found to be reduced mildly in the test formulation administered groups and the effect was found to be statistically non-significant.

In vivo colonic fluid transport was dramatically affected by acetic acid-induced colonic inflammation, changing from net absorption to net secretion (Table 1). Positive sign indicate the net absorption of colonic fluid in normal control group. This alteration in colonic fluid transport by acetic acid-induced colitis was reversed significantly by sulfasalazine ($p < 0.001$) and at some extent by pre-treatment with Chinnodbhavadi kwath but effect was statically non significant. The impairment of fluid absorption in colitic rats in present study primarily reflect the loss of viable epithelial surface in the early stage of colitis as reported in earlier studies²¹. Number of flavanoid compounds has been reported to inhibit intestinal motility and secretion²².

Oxidative stress has been implicated in the pathogenesis of ulcerative colitis in experimental animals²³. In the present study, the colitis was associated with significant increase in mucosal myeloperoxidase activity ($p < 0.01$) and lipid peroxidation ($p < 0.001$) that was parallel with the depleted level of glutathione ($p < 0.001$) and decreased activity of superoxide dismutase (SOD) ($p < 0.001$) when compared with normal control group values (Table 2). These changes considered in totality are indicative of oxidative stress. Measurement of myeloperoxidase activity

has been used as an indicator of neutrophil influx in to inflamed mucosa¹⁶. The reduction in colonic myeloperoxidase activity following treatment with Chinnodbhavadi kwath could be attributed to decreased neutrophil infiltration into the inflamed colonic tissue.

Glutathione deficiency signifies an excessive production of reactive oxygen species in acetic acid colitis control group²⁴. Treatment of rats with Chinnodbhavadi kwath significantly counteracted depletion of glutathione level and increasing the SOD level in colonic mucosa. A role for super oxide dismutase in the colitis is supported by protective effect of SOD mimetic enzymes in rodent models of colitis²⁵.

CONCLUSION

Thus based on the above presented activity profile it can be concluded that Chinnodbhavadi kwath produced moderate effect in the down regulation of inflammatory conditions of the colon in experimental colitis in rats. The test formulation has anti-oxidant activity which is probably due to presence of ascorbic acid, phenolic compounds including flavonoids. Additionally rich amount of tannin present in test formulation is known to affect the membrane integrity by its astringent property²⁶ which could be responsible for effects of Chinnodbhavadi kwath.

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Table 1: Effect of Chinnodbhavadi kwath on colonic damage score and fluid absorption in acetic acid-induced colitis in rats

Groups	Damage score	Fluid absorption ($\mu\text{l/h.g tissue}$)
Normal control	0	396.66 \pm 11.66
Colitis control	9.06 \pm 0.78 [†]	-74.33 \pm 03.48 [†]
Chinnod. Kwath (8.7 ml/kg)	8.95 \pm 0.46	-25.00 \pm 02.88
Sulfasalazine (100 mg/kg)	6.46 \pm 0.52 *	143.33 \pm 13.01 **

Results are expressed as mean \pm SEM of eight rats per group.

[†] $p < 0.001$ compared with normal control group.

* $p < 0.05$, ** $p < 0.001$ compared with colitis control group.

Table 2: Effect of Chinnodbhavadi kwath on colonic myeloperoxidase, lipid peroxidation, superoxide dismutase and glutathione content in acetic acid-induced colitis in rats

Groups	MPO (U/g tissue)	LPO (nmol MDA/g)	SOD (U/mg protein)	Glutathione (nmol/g tissue)
Normal control	52.09 ± 3.94	07.09 ± 0.40	3.10 ± 0.19	1668.64 ± 48.17
Colitis control	82.87 ± 4.46 [†]	11.86 ± 0.62 [‡]	1.53 ± 0.11 [‡]	889.94 ± 76.66 [‡]
Chinnod. Kwath (8.7 ml/kg)	72.73 ± 9.35	10.22 ± 0.96	2.29 ± 0.26	1321.02 ± 78.90*
Sulfasalazine (100 mg/kg)	54.29 ± 4.97*	09.81 ± 0.47	2.26 ± 0.24	1348.83 ± 128.2*

Results are expressed as mean ± SEM of eight rats per group.

[†]P < 0.01, [‡]P < 0.001 compared with normal control group.

*P < 0.01 compared with colitis control group.

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