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Ameliorative effects of Triphala on mucosal damage in rat model of ulcerative colitis

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In Ayurvedic practice, Triphala is widely used in gastric problems including constipation, large intestinal inflammation and colitis. The present research was planned to assess the ameliorative effects of Triphala formulations in reducing the magnitude and severity of ulcerative colitis. Triphala formulations prepared by mixing three fruits, *Haritaki* (*Terminalia chebula*), *Bibhitaki* (*Terminalia belerica*) and *Amlaki* (*Emblica officinalis*) in different ratios i.e., 1:1:1 (Triphala equal) and 1:2:4 (Triphala unequal) as per classical references. Wistar albino rats were administered with two ml of acetic acid (4% v/v) in intra-colonic lumen for induction of colitis. The efficacy of Triphala was measured on various parameters namely, *in vivo* fluid absorption in tied-off colon, ulcer score and colonic mucosal parameters. The degree of alteration in colonic fluid transport was significantly reversed by Triphala equal, Triphala unequal and sulphasalzine as standard drug. Triphala formulations significantly attenuated the nitric oxide (NO), myeloperoxidase (MPO) and lipid peroxidation (malondialdehyde MDA) levels in mucosa of rat colon. Pre-treatment with Triphala unequal formulation attenuated the severity of the colonic macroscopic damage score, histologic injury and counteracted the depletion of glutathione and superoxide dismutase activity hence, reduced the oxidative stress in colonic mucosa. Triphala unequal formulation has better protective effects. Outcomes of the present study reveal the usefulness of Triphala formulations in attenuating the colonic inflammation in experimental-induced ulcerative colitis.

Keywords: Acetic acid, Antioxidant property, Colonic fluid absorption, Triphala, Ulcerative colitis

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Ulcerative colitis is an inflammatory bowel disease (IBD), limited to colon characterized by inflammation and mucosal disruption. It is a chronic and relapsing disorder, pathogenesis of IBD remains unclear even though, decontrolled immune system accompanied by environmental and genetic factors affects the beginning and advancement of disease[1]. 5-aminosalicylic preparations are clinically used drug for treatment of IBD but having certain adverse effects, particularly when administered at higher dose or on repeated administration for longer time[2]. This provides alternate of using herbal formulations to treat IBD and associated symptoms[3].

Triphala is classified as rasayana (rejuvenator) and is said to promote longevity, well beings and immune system. The rasayana property of Triphala is well elucidated by Network Pharmacology plot which may suggest the multi-target approach of Triphala[4]. As per *Charaka*, Triphala is *Tridoshik rasayana,* having balancing effect on the three *Doshas,* a constitutional components human body[5]. It is widely practiced for GI tract disorders such as constipation, digestion related problems, poor food assimilation and large bowel inflammatory diseases[6]. It has anti-inflammatory[7], anti-oxidants[8] and intestinal cytoprotective[9] properties owing to its major phytoconstituents.

Triphala generally consists of dried pericarp of three fruits namely, *Terminalia chebula* Retz. (*Haritaki*), *Terminalia belerica* (Gaertn) Roxb. (*Bibhitaka*) and *Emblica officinalis* Gaertn. (*Amalaki* or Indian Gooseberry) fruits in equal ratio (1:1:1)[10] while some authors advocate formulation using 1:2:4 ratio of *Haritaki, Bhibhitaka* and *Amalaki*[11,12]. Previous researches also confirmed the role of customary prepared Triphala formulation in dextran sodium

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sulphate[13] and 2,4,6-trinitrobenzene sulfonic acid (TNBS)[14] induced colitis in rats. However, probable modulatory role of Triphala formulations prepared as per classical concepts by mixing the fruits in different ratios has not been yet verified in colon inflammation in acetic acid-induced colitis in rats. Therefore, the present work was planned with an aim to assess the efficacy of Triphala formulations prepared with two different ratios on experimental colitis in rats.

Materials and Methods

Drugs and chemicals

T. chebula (*Haritaki*), *T. belerica* (*Bibhitaka*) and *E. officinalis* (*Amalaki*) fruits were collected from its natural habitat, authenticated and specimens preserved in the Institute. Triphala equal and Triphala unequal formulations were prepared using equal ratio (1:1:1) and unequal ratio (1:2:4) of *Haritaki, Bhibhitaka* and *Amalaki.* For biochemical parameters, chemicals and reagents used were of analytical reagent (AR) grade.

Animals

Healthy wistar albino rats weighing 200±20 g were selected in the research study. Temperature and relative humidity maintained as per standard husbandry conditions and illumination controlled to sequence of 12 h light and dark. Pelleted rat/mice diet "Amrut" brand and purified drinking water provided *ad libitum*. An approval for the study protocol was obtained from Institutional Animal Ethics Committee (IAEC/LMCP/282).

Experimental design

Total 40 rats were selected based on body weight and randomly distributed in to five groups (n=8) in way to minimum variation in mean body weight between groups. Normal control group (I) and Colitis control group (II), treated with vehicle as 1% (w/v) carboxymethyl cellulose solution in water. In a previous work, 5 mL/kg, 1% (w/v) carboxymethyl cellulose has been reported to have no influence on experimental-induced colitis in rats[15]. Drug treated groups (III and IV) received Triphala equal (540 mg/kg) and Triphala unequal (540 mg/kg) formulations respectively for 7 days. Standard drug group (V), sulafasalazine (100 mg/kg) administered on 5th, 6th and on 7th day, 1 h prior to induction of ulcerative colitis[16].

Rats were deprived of food for overnight but allowed to drinking water before induction of acetic acid-induced colitis as explained by MacPherson and Pfeiffer[17]. Briefly, to fasted albino rats, under light ether anesthesia, medical grade polyethylene tube (2 mm diameter) inserted in to colon and the tip of tube reach to 8 cm proximal to anus. 4% (v/v) acetic acid solution prepared in distilled water and 2 mL was instilled in to lumen of colon, thereafter acidic solution was neutralized after contact of 10 seconds. In control group, rats instilled with phosphate buffer saline in same volume. On 8th day after colonic fluid absorption study, rats were sacrificed. Colonic segment was excised from each albino rat, cleaned of extra tissue, rinsed with saline and further used for macroscopic damage, biochemical parameters and histopathological studies.

Colonic fluid absorption- In vivo study

The tied-off colon procedure[18] was performed in anesthetized rats (urethane, 1.2 g/kg body weight, ip) on warm table. Lapratomy was done and 2 cm distal colon from the cecocolic junction was ligated. The colonic stuffs were washed away out with of saline (20+20 ml, 37ºC), then dried off with injecting air (20+20 mL). A second ligature was placed 1 cm above the rectal plaque. 1 mL tyrode solution (37ºC) was injected to colon proximal end and then ligature closed. The opening was sutured after placing the colon in abdominal cavity. After one hour of experiment, rat was sacrificed and tied off colon was excised. The residual fluid was collected and accurately weighed. The change in the volume (µL/h.g wet tissue) was measured and subtracted from its initial weight; positive values indicate the absorption while negative values treated as secretion. As per previous study, colon tissue biochemical parameters were not altered after colonic fluid absorption study[19].

Colonic macroscopic damage

Colonic segment was dissected, adherent tissue removed, split out longitudinally and rinsed with chilled saline. Macroscopic damage score by independent investigator was given ranging from 0 to 10 scale[20] depending upon severity of colonic destruction in each rat. The wet colon weight, length and weight/length ratio was determined. About 2 g load was applied to the colon for length dimension.

Biochemical analysis

Longitudinal segments of colon stored at -20ºC immediately after excision and afterwards subjected

to biochemical parameters. The measured parameters were protein[21], myeloperoxidase (MPO) activity and expressed as units/g tissue (one unit of activity calculated to degrading 1 µmol peroxide/min at 37°C)[22], glutathione[23], nitric oxide (NO) was measured by the acidic Griess reaction[24], superoxide dismutase activity expressed as units/mg protein[25] (one unit of activity calculated to inhibition of reaction rate by 50% per minute) and lipid peroxidation (LPO) evaluated by measuring quantity of malondialdehyde (MDA) and expressed as nmol of MDA/g tissue[26].

Statistical analysis

The results are presented as mean ± SEM (standard error of mean) with n = 8 animals in each group. Statistical analysis was done using ANOVA (One way analysis of variance) followed by Dunnett's multiple 't' test to compare the mean of quantitative variables between groups. The minimal standards of significance was considered as $p<0.05$.

Results and Discussion

Colonic administration of acetic acid produced experimental colitis in albino rats is one of the well-known and consistent models which have similarity to acute inflammation in human intestine[27]. The rat colon instilled with acetic acid showed symptoms of colitis with severe inflammation in colonic mucosa, ulcerations and lesions with blood spots, as shown by increase in damage score on macroscopic evaluation. Colon wet weight increased while length decreased as revealed by significant increase in weight/length ratio (mg/cm) in colitis control group (Table 1). Prior treatment of Triphala formulation having unequal ratio and sulfasalazine reduced the severity of macroscopic ulceration and colonic damage score at significant level compared to colitis control group.

Ulcerative colitis accompanying inflammation of mucosa associated with symptoms of diarrhea. Therefore, the diminishing *in vivo* colonic absorption of fluid mainly reveals the initial phase of ulcerative colitis with damage of epithelial lining[20]. In normal control group, there was net absorption of fluid in tied-off colonic loop represented by positive sign. However, inflammation of colonic mucosa in acetic acid colitis group intensely affected the *in vivo* colonic fluid absorption to net secretion represented by negative sign (Table 1). Both Triphala formulations and sulfasalazine significantly attenuated the acetic acid-induced alteration in fluid transport and exhibited net absorption of colonic fluid. In previous studies, many flavonoids are stated to reduce the intestinal secretion and its motility[28] and well known flavonoid quercitrin also reversed the mucosal injury after chronic diarrhea in rats[29]. Triphala having good amount of flavanoid may be responsible for its protective effects on colonic mucosal membrane[13].

Acetic acid-induced colitis in experimental model associated with oxidative stress in inflammed colonic mucosa[30]. Present study showed inflammed colonic mucosa associated with significant increase in MDA, MPO and NO in colonic mucosa along with reduced amount of antioxidant parameters such as superoxide dismutase activity and glutathione level in comparison with control group (Table 2). Increased MPO activity suggests the neutrophil recruitment in damaged colonic mucosa of rats[31]. Significant decrease in colonic MPO activity in both Triphala formulations treated groups may be attributed to its anti-inflammatory effect[7].

Triphala has powerful antioxidants effect which may reduce the oxidative stress in inflammed colonic mucosa colon[14] as revealed by significant reduction in the thiobarbituric acid reactive substances such as MDA which is a good indicator of LPO[26]. The free radical scavenging activity of Triphala may be due to presence of well-known phytoconstituents particularly gallic acid (phenolic compound)[32]. In another study, flavanoid extracted from *Emblica officinalis* reported to reduce the generation of MDA in albino rats[33].

Table 1 — Effect of Triphala formulations on damage score, *in vivo* fluid absorption and weight and length ratio in albino rats

Groups	Damage score	W/L ratio (mg/cm)	*In vivo* fluid absorption (pL/h.g tissue)
Control	0	95.61±3.64	396.66±11.66
Colitis control	9.06±0.78*	135.44±3.08*	-74.33±03.48*
Triphala equal	8.63±0.73	130.93±1.82	20.33±17.66 #
Triphala unequal	6.41±0.78 #	125.80±3.01	68.33±44.75 ##
Sulfasalazine	6.46±0.52 #	122.64±2.39 #	143.33±13.01 ###

Data: Mean ¶ SEM, n=8 per group

*p<0.01 *vs* control group; # p<0.05, ## p<0.01 *vs* colitis control group (One way ANOVA followed by Dunnett's test)

Table 2 — Effect of Triphala formulations on colonic tissue parameters in albino rats

Groups	MPO (Unit/g tissue)	LPO (nmol MDA/g tissue)	SOD (Unit/mg protein)	NO (pmol/g tissue)	Glutathione (nmol/g tissue)
Control	52.09±3.94	7.09±0.40	3.10±0.19	1.028±0.054	1668.64±48.17
Colitis control	82.87±4.46 *	11.86±0.62*	1.53±0.11*	1.516±0.052*	889.94±76.66*
Triphala equal	61.90±4.18$^{\#}$	9.40±0.62$^{\#}$	1.81±0.25	1.361±0.032$^{\#}$	1001.19±77.67
Triphala unequal	57.84±3.65$^{\#}$	9.40±0.62$^{\#}$	2.53±0.35$^{\#}$	1.352±0.036$^{\#}$	1223.68±41.25$^{\#}$
Sulfasalazine	54.29±4.97$^{\#}$	9.81±0.47	2.26±0.24	1.343±0.031$^{\#}$	1348.83±128.2$^{\#\#}$

Data: Mean ¶ SEM, n=8 per group

* $p<0.01$ *vs* control group; $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$ *vs* colitis control group (One way ANOVA followed by Dunnett's test)

Thus, the findings of this research work are in conformity with previous annotations. The reduction in NO level in inflamed mucosa by both Triphala formulations also confirm with the earlier research work[34]. The reduction in effect of NO may be due to the presence of ascorbic acid which is available in rich amount in *Amalaki*[35].

Decrease in glutathione content suggests the excessive generation of free radicals in mucosa of ulcerative colitis in rats[36]. Triphala unequal formulation significantly restored the glutathione and superoxide dismutase in colonic mucosa of albino rats. Earlier study also supported the protective role of superoxide dismutase during colitis in rodent model[37]. Flavonoids and tannoid principles of Triphala formulation may be responsible for increased level of antioxidant parameters, superoxide dismutase and glutathione which may protect against free radicals-induced changes in colon of rats[33].

Emblica officinalis is well known for its anti-oxidant and free radical scavenging effects owing to enhanced ascorbic acid, gallic acid, ellagic acid and flavanoid contents. *T. belerica* contains high quantity of phenolics particularly gallic acid along with ellagic acid which is responsible for its antioxidant effects[8]. Earlier report showed that, ellagic acid prevents the experimental-induced colitis in rats due to its free radical scavenging effects[38]. Triphala formulation is very popular for its tannin rich content and due its astringent property may protect the membrane integrity of epithelial lining in colon of rats[39].

The present data indicates that, flavonoid present in Triphala with other bioactives as ascorbic acid, tannoid principles and phenolics may have contributed significantly to the anti-oxidant and beneficial effects observed against experimental-induced colitis in albino rats. Thus, Triphala formulations, especially those with unequal ratio, can be considered as an interesting approach in attenuating the colon inflammation in experimental ulcerative colitis in albino rats.

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Conflict of Interest

Authors declare no competing interest.

Authors' Contributions

MN: Experimental studies and original draft; PN: Experimental studies and writing support; BR & SG: Experimental studies and guidance

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