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Pharmacological study

Effect of *Triphala* on dextran sulphate sodium-induced colitis in rats

Vinay Rayudu, Akondi B. Raju[1]

Department of Pharmacology, St. Peters Institute of Pharmaceutical Sciences, Vidyanagar, Hanamkonda, Warangal, Andhra Pradesh, India, [1]Department of Pharmacology, Ibn Sina National College for Medical Studies, Al Mahjar, Jeddah, Kingdom of Saudi Arabia

Abstract

Background: Herbal products from Ayurveda were always in the forefront in providing leads to new drug discovery. *Triphala*, an ancient Ayurvedic herbal formulation comprises of equal portions of *Amalaki, Bibhitaki* and *Haritaki* and is used extensively for constipation, as an anti-inflammatory, analgesic, anti-arthritic, hypoglycemic and an anti-aging agent. **Aim:** To evaluate the effect of *Triphala* on dextran sulphate sodium induced colitis in rats. **Materials and Methods:** Present study carried out in total five groups (n = 6 in each group); first group served as normal, second group control, third group standard control and remaining two as test drug groups. Mesalzine was used as a standard drug for comparison. Two doses (150 mg/kg and 300 mg/kg) of *Triphala* were given as treatment for two separate groups of colitis rats for 7 days. C-reactive protein, superoxide dismutase, catalase, malondialdehyde levels were evaluated and histological study of the distal colon was conducted. **Results:** The colitis rats treated with higher dose of *Triphala* (300 mg/kg) exhibited normal parameters similar to normal control group animals, which is on par with standard drug mesalzine effect. **Conclusion:** The results suggest that *Triphala* (300 mg/kg) has a considerable and reliable effect in reducing colitis in rats. This effect can be attributed to its antioxidant activity and well presence of flavonoids.

Key words: Antioxidant, colitis, dextran sulphate sodium, inflamatory mediators, *Triphala*

Introduction

Triphala is an Ayurvedic herbal formula consisting of equal parts of *Amalaki* (*Phyllanthus emblica* Itis.), *Bibhitaki* (*Terminalia bellirica* Roxb.) and *Haritaki* (*Terminalia chebula* Retz.)[1] *Triphala* is being employed in conditions like headache, dyspepsia, constipation, ascites and leucorrhoea and as a blood purifier. It is reported to possess anti-inflammatory, analgesic, anti-arthritic, hypoglycemic and anti-aging properties.[2] Major phyto-constituents in *Terminalia belerica* are ellagic and gallic acid. *Emblica officinalis* has several gallic acid derivatives including epigallocatachin gallate. Major ingredient in the *Terminalia chebula* is gallic acid, which is also found to be an antioxidant.[3]

Address for correspondence: Dr. Akondi Butchi Raju, Asst. Prof. and Head, Department of Pharmacology, Ibn Sina National College for Medical Studies, Post Box No. 31906, Al Mahjar, Jeddah - 21418, Kingdom of Saudi Arabia.
E-mail: drraju2020@gmail.com

Colitis refers to an inflammation of the colon and is often used to describe an inflammation of the large intestine (colon, caecum and rectum) and its symptoms are abdominal pain, loss of appetite, fatigue, diarrhea, cramping, urgency and bloating.[4] Currently, there is no effective therapy to cure the disease, but the mainstream treatment depends on reduction of the abnormal inflammation in the colon lining. Herbal drugs from Ayurveda have already proved to be the important leads for drug development. As *Triphala* is reputed for colon cleansing, management of digestion problems, large intestine inflammation and ulcerative colitis in Ayurveda, in the present study, the effect of *Triphala* in the treatment of colitis in rats was evaluated to provide scientific evidences.

Materials and Methods

Chemicals

Dextran sulfate sodium (Batch No. T-835310) was purchased from SRL Pvt. Ltd., Mumbai. Capsules of *Triphala* (Batch No. F3710158) containing *Triphala* extract manufactured by "The Himalaya Drug Company", Bangalore was purchased. Assay kit for estimation of C-reactive protein (CRP) in serum

was obtained from Tulip diagnostic limited. All other chemicals used were of analytical grade.

Experimental animals

A total of 36 Female Wistar rats weighing 150-180 g were procured from Sanzyme Ltd., Hyderabad, India and used for the experiment. They were housed in cages with a maximum of 6 rats and were maintained in an air conditioned room (25 ± 2°C) with Light: Dark cycle 12:12 h and relative humidity range of about 55 ± 10%. They were fed with semi purified basal diet and drinking water *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for a week before the start of the experiment. All experimental procedures were conducted in approval with Institutional Animal Ethics committee (Reg. No. 1126/bc/07/CPCSEA and approval no. 15/SPIPS/IAEC/2012) for the care and use of animals and were strictly followed as per CPCSEA guidelines throughout the study.

Phytochemical investigation

Phytochemical tests of flavonoid by using lead acetate test, alkaline reagent test, ferric chloride test and schinoda test for phenolics as carried out using standard procedures.[2]

Acute toxicity studies

Acute toxicity study was conducted for test drug according to the OECD guidelines No: 425 and changes in behavioral responses were not observed in animals (n = 6) upon oral administration of *Triphala* at a limit test dose of 2000 mg/kg. All animals survived the test and the LD50 was declared above 2000 mg/kg. No mortality was observed in the late phase of the study for 14 consecutive days. Thus, a dose of 150 mg/kg and 300 mg/kg were taken for the study.[5]

Experimental design

Animals were randomly divided into five groups of six animals each (n = 6). Experimental colitis was induced in rats (Group II-V) by oral administration of 3% dextran sulfate sodium (DSS) solution as drinking water *ad libitium* for 7 days. Group-I consists of normal rats which are free of drugs. As Group-II received only DSS, it was considered as control group. Group-III consists of rats treated with Mesalazine 100 mg/kg/orally, whereas animals of Group-IV and Group-V were treated with *Triphala* 150 mg and 300 mg/kg/orally respectively. All rats were fed with normal diet and drinking water *ad libitium* for the entire study.[6]

All rats were fasted overnight, but had free access to water after the last dose of administration and rats were sacrificed after 24 h of their last dose. Animals were anaesthetized and blood was collected from retro-orbital puncture to estimate various parameters and then animals were sacrificed by cervical dislocation. Abdomen was opened by midline incision and liver and colon were collected for estimation of various parameters.

Assessment of biochemical parameters

The tissue homogenates of liver and colon were prepared by homogenizing 1 g of tissue in sodium phosphate buffer solution. The enzyme superoxide dismutase (SOD) was determined in tissue homogenate using photo-oxidation method.[7,8] The amount of lipid peroxidation end products present in the tissue homogenate was estimated by the thiobarbituric acid reactive substances method, which measures the malondialdehyde (MDA) reactive products spectrophotometrically.[9] The estimation of the liver Catalase activity was done based on the ability of catalase to oxidize hydrogen peroxide.[10] Estimation of amount of glutathione in blood was done on the principle of formation of glutathione, a colored complex with DTNB, which was measured spectrophotometrically. Estimation of colonic glutathione peroxidase (GPx) enzyme activity which is found in cytoplasmic and mitochondrial fractions of cells is done by reaction of GPx on lipid hydroperoxide substrates that are released from membrane phospholipids by phospholipase A2.[11] CRP levels in blood, which is synthesized in the liver is estimated by using CRP estimation kit.[11]

Colon weight/length ratio (mg/cm)

The collected colon from sacrificed animals were gently flushed with saline, placed on an ice-cold plate, cleaned of fat and mesentry and blotted on filter paper to dry. Each colon was weighed and its length was measured and the weight/length ratio (mg/cm) was determined.

Histopathological analysis

Piece of colon was fixed in 10% natural buffered formalin solution, embedded in paraffin, cut into tissue sections and stained with hematoxylin and eosin. The stained sections were examined by light microscope for evidence of colitis using the following criteria: Presence of inflammatory cell infiltration, presence of crypt abscesses, crypt distortion and regenerative changes in the form of nuclear enlargement and increased mitotic activity, cases treated with drugs were examined for histological signs of resolution in × 10 optical zoom[12] [Table 1].

Statistical analysis

Results are expressed as mean ± standard error of the mean Statistical analysis was performed using an unpaired t-test and ANOVA followed by the Dunnett's multiple comparison tests.

Table 1: Histological score of colitis induced by dextran sulphate sodium

Histological feature	Score	Description
Loss of epithelium	0	None
	1	0-5% loss of epithelium
	2	5-10% loss of epithelium
	3	Over 10% loss of epithelium
Crypt damage	0	None
	1	0-10% loss of crypt
	2	10-20% loss of crypt
	3	Over 20% loss of crypt
Depletion of goblet cells	0	None
	1	Mild
	2	Moderate
	3	Severe
Infiltration of inflammatory cells	0	None
	1	Mild
	2	Moderate
	3	Severe

$P < 0.05$ was considered to be significant. Statistical analysis was performed using Graph Pad Prism 5 software (manfactured by Graph Pad Prism Inc. USA).

Results

Phytochemical tests of *Triphala* showed positive result for lead acetate test, alkaline reagent test, ferric chloride test and test for phenolics and thereby confirmed the presence of flavonoids.

As shown in Table 2, the *Triphala* (300 mg/kg) exhibited a significant ($P < 0.05$) effect in preventing the increase of the levels of CRP in blood in DSS induced rats when compared with lower dose of *Triphala* (150 mg/kg). The high dose of *Triphala* (300 mg/kg) prevented the decrease of SOD levels; catalase in colon in DSS induced rats [Table 2] more effectively when compared with low dose of *Triphala* (150 mg/kg). The effect of high dose of *Triphala* (300 mg/kg) is comparable with that of standard drug Mesalazine (150 mg/kg).

The amount of lipid peroxidation was high in control animals than in normal animals. Lipid peroxidation was inhibited in *Triphala* (300 mg/kg) treated animals when compared with that of control animals and the results were significant [Table 3]. *Triphala* (150 and 300 mg/kg) exhibited no effect on glutathione levels when compared with control group. Standard drug Mesalazine (100 mg/kg) had a significant effect in preventing the decrease of glutathione levels in blood. Both *Triphala* (150 and 300 mg/kg) groups exhibited a significant effect in protection of cells against oxidative damage by preventing the fall of GPx activity. The results were comparable with that of the standard drug [Table 3].

The pathologic processes in the colon were recognized macroscopically as a progressive colonic thickening and shortening. The colonic weight to length ratio (mg/cm) was significantly higher in control animals (112.1 ± 2.868) than in normal animals (80.99 ± 1.712). The ratio was decreased in *Triphala* (300 mg/kg) treated group [Table 3].

When compared with control group *Triphala* at 300 mg/kg showed a significant protective effect in preventing damage of the colon. Standard drug Mesalazine (100 mg/kg) and *Triphala* (300 mg/kg) showed an equal score of 1.33 ± 0.210 [Table 3].

Photomicrographs of hematoxylin-stained sections of rat distal colon

Histological analysis of photomicrographs of hematoxylin and eosin stained sections of rat colon in normal group animals showed intact epithelial cells. The colon exhibited intact goblet cells and no inflammatory cells infiltration [Figures 1a and 2c].

Table 2: Effect of *Triphala* on CRP, SOD, catalase and MDA levels

Treatment groups	CRP (mg/dl)	SOD (U/mg protein)	Catalase (U/mg protein)	MDA (nmol/g tissue)
Group-I Normal	1.80±0.26	26.09±3.08	15.61±0.20	2.68±1.00
Group-II Control	12.00±2.40#	4.87±1.46#	0.70±0.25#	8.89±0.57#
Group-III Standard	3.80±1.25*	19.01±1.62*	4.55±1.07	4.73±1.03*
Group-IV Test drug (150 mg/kg)	6.00±1.20	11.23±0.73	4.095±1.04	5.89±0.28*
Group-V Test drug (300 mg/kg)	2.60±0.48*	19.00±0.80*	7.76±1.67*	4.92±0.16*

Group-I: Normal (no treatment), Group-II: Control (3% DSS alone), Group-III: Standard (100 mg/kg Mesalazine along with 3% DSS), Group IV and V (*triphala* 150 mg/kg and 300 mg/kg along with 3% DSS orally). Results represent the mean±SEM from six rats. #$P<0.05$ compared with normal group, *$P<0.05$ compared with control group. DSS: Dextran sulfate sodium, SEM: Standard error of the mean, CRP: C-reactive protein, SOD: Superoxide dismutase, MDA: Malondialdehyde

Table 3: Effect of *Triphala* on glutathione, GPx, colon weight/length ratio and histological score

Treatment groups	Glutathione	GPx	Colon weight/length ratio (mg/cm)	Histological score
Group-I Normal	0.47±0.03	192.90±4.35	80.99±1.712	0.00±0.00
Group-II Control	0.03±0.007#	46.30±1.87#	112.1±2.868#	3.00±0.00#
Group-III Standard	0.14±0.03*	136.30±2.20*	93.09±1.857*	1.33±0.210*
Group-IV Test drug (150 mg/kg)	0.05±0.007	100.30±2.20*	103.2±2.481*	2.00±0.365*
Group-V Test drug (300 mg/kg)	0.05±0.009	126.00±2.20*	90.05±3.27*	1.33±0.210*

Group-I: Normal (no treatment), Group-II: Control (3% DSS alone), Group-III: Standard (100 mg/kg Mesalazine along with 3% DSS), Group-IV and V (*Triphala* 150 mg/kg and 300 mg/kg along with 3% DSS orally). Results represent the mean±SEM from six rats. #$P<0.05$ compared with normal group, *$P<0.05$ compared with control group. DSS: Dextran sulfate sodium, SEM: Standard error of the mean, GPx: Glutathione peroxidase

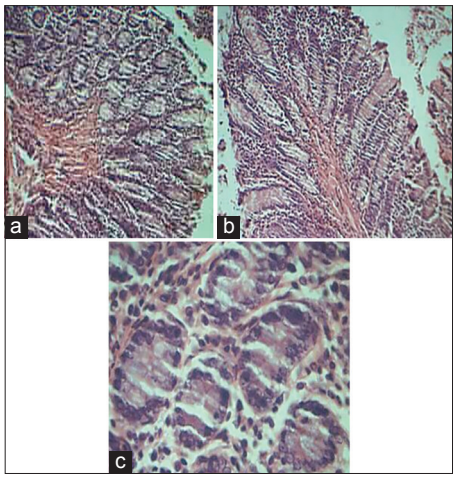

Figure 1: Normal group. (a and b) Represent photomicrographs of H and E stained sections of rat colon showing intact epithelial surface; ×10. (c) Showing the normal appearance of goblet cells; ×40.

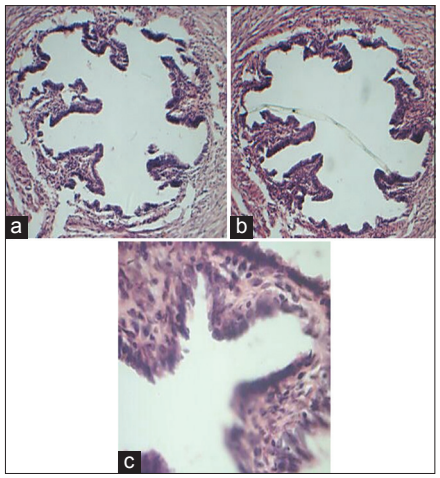

Figure 2: Control group. (a and b) Represent photomicrographs of H and E stained sections of rat colon showing distorted epithelial surface; ×10. (c) Showing the complete loss of goblet cells and inflammatory cells infiltration; ×40.

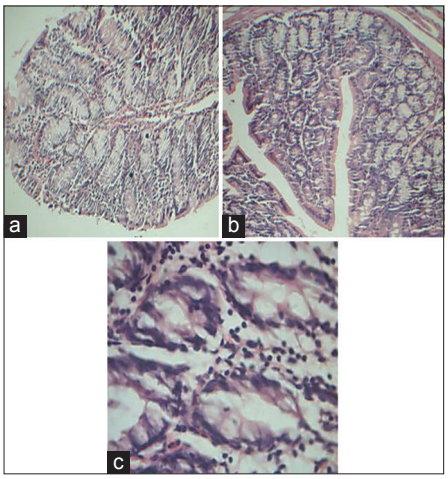

Figure 3: Standard group. (a and b) Represent photomicrographs of H and E stained sections of rat colon showing protection of mesalazine (100 mg/kg) against distortion of epithelial surface; ×10. (c) Showing the intact goblet cells and low inflammatory cells infiltration; ×40.

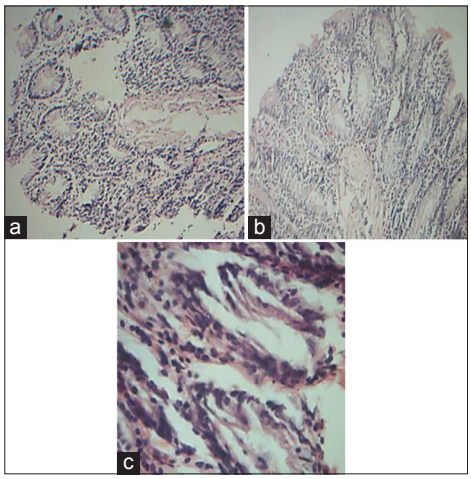

Figure 4: *Triphala* (150 mg/kg) treated group. (a and b) Represent photomicrographs of H and E stained sections of rat colon showing a moderate protection of *Triphala* (100 mg/kg) against distortion of epithelial surface; ×10. (c) Showing the semi intact goblet cells and low inflammatory cells infiltration; ×40.

Animals treated with DSS alone (control group) had a marked loss of epithelium of colon, crypt damage, goblet cell depletion and inflammatory cells infiltrations [Figure 2a-c]. In standard group animals showed intact epithelial cells when compared to control group [Figure 3a–c]. The colon exhibited intact goblet cells and less inflammatory cells infiltration when compared to control group on treatment with Mesalazine. In *Triphala* (150 mg/kg) treated group moderately destructed epithelial cells were found when compared to control group. The colon exhibited intact goblet cells and moderately infiltrated inflammatory cells [Figure 4a–c]. In *Triphala* (300 mg/kg) treated group animals showed intact epithelial cells. The colon exhibited intact goblet cells and less inflammatory cells infiltration [Figure 5a–c], which is comparable with that of the standard drug treated group.

Discussion

Colitis is an inflammation of colon which is often used in medical context to describe an inflammation of the large intestine (colon, caecum and rectum). Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, etc., and compounds that can scavenge free radicals have great potential in ameliorating these disease processes.[13] Antioxidants thus play an important role to protect the human body against tissue damage by reactive oxygen species.

DSS is a polyanionic derivative of dextran, produced by esterification with chlorosulfonic acid. DSS is a sulfated polymer and induces colitis in rodents,[6,14] and rat DSS colitis resembles human ulcerative colitis both histologically and topologically.[15] The exact mechanism

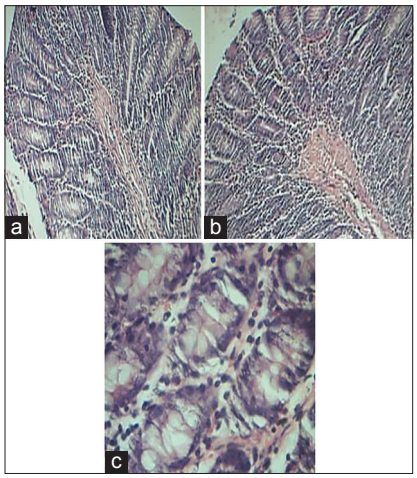

Figure 5: *Triphala* (300 mg/kg) treated group. (a and b) represent photomicrographs of H and E stained sections of rat colon showing a marked protection of *Triphala* (100 mg/kg) against distortion of epithelial surface; ×10. (c) Showing the intact goblet cells and low inflammatory cells infiltration; ×40.

through which DSS initiates colitis is unknown but one possible mechanism may be direct alteration of gut permeability. Tight junction proteins such as zona occludens-1 were directly reduced by DSS as early as day one, leading to increased permeability by day three, changes that preceded colonic inflammation,[12,16,17] DSS may also cause concentration-dependent direct cytotoxicity on colonic mucosa, which leads to alteration of integrin-α4 and M290 subunit levels on epithelial cells disrupting their interaction with the γδ-intraepithelial T cells which has mucosal protective action.[18]

In the animal group treated with DSS, an increase in the oxidative stress was observed indicated by the higher MDA and CRP levels and as well as a decrease in SOD, catalase, GSH-Px activity which might be responsible for the tissue damage and development of inflammation.

The results suggest that *Triphala* (300 mg/kg) has a considerable and reliable effect in reducing colitis in rats. This effect can be attributed to its antioxidant activity and well presence of flavonoids. The above study also suggests the positive role of *Triphala* in suppression of inflammatory mediators leading to the suppression of colitis. Earlier studies on *Triphala* proved it as anti-inflammatory, cytoprotective and immunomodulatory in nature and support the usage of *Triphala* in the treatment of colitis.[19-21]

Conclusion

Based on the findings it can be concluded that *Triphala* formulation has an anti-oxidant and anti-inflammatory action in reducing colitis in rats. These activities may be due to the presence of flavonoids. Thus, the *Tripahala* can be effectively used in the treatment of colitis, however further studies are required.

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हिन्दी सारांश

डेक्स्ट्रान सल्फेट सोडियम द्वारा चूहों में उत्पन्न कोलाइटिस पर त्रिफला का प्रभाव

विनय रायडू, अंकोडी बी. राजू

हर्बल उत्पादन नई दवाओं की खोज के सुराग प्रदान करने के लिए हमेशा आगे है। त्रिफला, एक प्राचीन आयुर्वेदिक योग है, जिसमे आमलकी, बिभीतकी और हरीतकी बराबर भागों मे शामिल हैं और कब्ज के लिए, एनाल्जेसिक, हायपोग्लायसेमिक, और रसायन के रुप में बड़े पैमाने पर इस्तेमाल किया जाता है। वर्तमान अध्ययन चूहों में डेक्सट्रॅान सल्फेट सोडियम प्रेरित कोलाइटिस पर त्रिफला का प्रभाव देखने हेतु किया गया। इस अध्ययन मे कुल ३० चूहों को, ५ वर्गों (एन = ६) में आवंटित किया गया। प्रथम वर्ग नॉर्मल कंट्रोल, व्दितीय निगेटीव कंट्रोल, तृतीय स्टँडर्ड कंट्रोल, और शेष २ वर्ग त्रिफला उपचारित के रुप में आवंटित किये गये। वर्ग २ से ५ के चूहों में ३% डेक्सट्रॅान सल्फेट सोडियम का द्रव पिलाकर कोलाइटिस प्रेरित किया गया। वर्ग ३ मे मानक दवा Mesalzine १०० मि.ग्रा./कि.ग्रा. मात्रा मे दी गयी। त्रिफला की दो खुराक वर्ग ३ एवम् ४ मे १५० मि.ग्रा./कि.ग्रा. और ३०० मि.ग्रा./कि.ग्रा. मात्रा मे दी गयी। C-reactive protein, superoxide dismutase, catalase, malondialdehyde (MDA) के स्तर का मूल्यांकन तथा बृहदान्त्र के ऊतकविकृतिविज्ञान का अध्ययन किया गया। विविध मापदण्डों में त्रिफला की उच्च मात्रा (३०० मि.ग्रा./कि.ग्रा.) का प्रभाव नॉर्मल कंट्रोल वर्ग के समान तथा मानक दवा Mesalzine के बराबर पाया गया। इन परिणामों से यह सिद्ध होता है कि त्रिफला (३०० मि.ग्रा./कि.ग्रा.) चूहों मे कोलाइटिस के इलाज मे महत्त्वपूर्ण एवम् विश्वासनीय औषधि है। यह परिणाम त्रिफला कि एण्टी–ऑक्सीडण्ट गतीविधीयाँ तथा फ्लेवेनॉईडस की उपस्थिति दर्शाता है। त्रिफला शुद्धता, सुरक्षा और प्रभावकारिता, भविष्य के अध्ययन का विषय है।