Original Article

Protective Effect of A_{2B} Receptor Antagonist (TRP 1) on Acetic Acid Induced Ulcerative Colitis in Rats: *in vitro, in vivo* and *in silico* Methods

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ABSTRACT

Aim: Present study was elucidate the protective effect of pyridinone derivatives such as 7-amino-5-oxo-2- Phenyl-5H, 8H-dihydro-[1, 2, 4] triazolo [1, 5-α] pyridine - 6-carbonitril (TRP 1) by *in vitro, in vivo and in silico.* **Methods:** Radioligand binding assay was performed on human adenosine receptors (A_{2B}) and assess A_{2B} antagonist effect by adenylyl cyclase activity. *In vitro* study was carried out to determine the neutralize capacity against DPPH*, NO*, SO*, LPO* free radicals. TRP 1 at the doses 1 mg/kg bd.wt. and 10 mg/kg bd.wt p.o, was administered consecutively for 14 days in albino rats. Ulcerative colitis was induced with single dose of 2 ml of 3% acetic acid intrarectal on 14th day in treated rats. At the end of treatment, colonic tissue was collected and subjected for estimation of macroscopic score, glutathione, catalase, MPO and inflammatory parameters such as IL 1β, TNF α and IL 6. *In silico* study was carried out to evaluate the binding energy and IC_{50} toward IL 1β, TNF α and IL 6. **Results:** TRP 1 was antagonized the A_{2B} receptors at the concentration of 30000 nM. *In vitro* study was revealed that TRP1 (1 mg/ml) was significantly neutralizes the free radicals of DPPH*, SO*, NO*and LPO*. In *in vivo* studies, intrarectal administration of acetic acid caused significantly (***P<0.001) increased macroscopic score, colon weight, colonic MPO, IL 6, IL 1β and TNF-α (*P<0.05), while TRP 1 treated colitis rats antioxidants system such as GSH (**P<0.01), catalase (*P<0.05) activity was significantly improved, decreases inflammatory mediators such TNF α (*P<0.05), IL 1β (**P<0.01) , IL 6 (**P< 0.01) and also suppresses the MPO activity (*P<0.05). *In silico* study was reported that the IC_{50} of TPR 1 against IL 1β, IL 6 and TNF-α was 7.5 mM, 28.65 mM and 45.87 mM respectively. **Conclusion:** Our data demonstrated that the TRP 1 treatment improved clinical score in acetic acid induced colitis in rats. It also inhibited the proinflammatory cytokine IL-6, IL 1β and TNF α and improvements of antioxidant in colitis rats through A_{2B} receptor antagonist property.

Key words: 7-amino-5-oxo-2- phenyl)-5H, 8H-dihydro-[1,2,4] triazolo [1,5-α] pyridine - 6-carbonitril (TRP 1), Ulcerative colitis, Acetic acid, Myeloperoxydase (MPO), Glutathione (GSH), Catalase, TNF α, IL 1β and IL 6.

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INTRODUCTION

IBD, including Crohn's disease (CD) and ulcerative colitis (UC), is a lifelong disabling gastrointestinal disease.[1,2,3] Although etiology of inflammatory bowel disease (IBD) is unknown it appears that an abnormal response of the mucosal innate immune system to luminal bacteria may trigger inflammation which is perpetual by disregulation of cellular immunity[4,5,6] and imbalances between proinflammatory cytokines, such as

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TNF-α, IFN-γ, IL-1β, IL-6, and IL-12, and anti-inflammatory cytokines like IL-4, IL-10, IL-11. Therapeutic agents for IBD which include anti-inflammatory agents such as 5-aminosalicylates (5-ASA) and corticosteroids along with some immunomodulators like azathioprine, 6-mercaptopurine were used. However, treatments are associated with severe adverse events including diarrhea, cramps, abdominal pain accompanied by fever and high blood pressure. Thus, there is a need to develop new therapeutic options with low toxicity and minimal side effects. In the search for novel therapeutic options, increasing attention is being paid to the adenosine system and its involvement in the pathophysiology of IBDs. Extracellular adenosine binds to adenosine receptors (AR) 1, 2A, 2B and 3, all of which are expressed on the surface of immune cells. Low level expression of A_1R is demonstrated in small intestine. $A_{2B}R$ are highly expressed in the cecum and colon, esophagus, stomach, and jejunum but appears to be absent in the ileum.[7,8] Inflammatory mediators like TNFα, IL6 are increased in the intestinal mucosa, serum and stools of patients with IBD through up regulation and over expression of A_{2B} receptors.[9,10,11,12,13] Past scientific studies are supported that fused pyridinone ring derivatives have been found to versatile pharmacophore with wide range of useful biological activities due to antagonize adenosine receptors[14,15] and ameliorated the inflammation.[16,17,18] Hence A_{2B} Rare great deal of interest, its primary molecular target and its mechanism of action remain to be clarified. In the present study was evaluated the protective effect of pyridinone derivatives like 7-amino-5-oxo- 2- phenyl -5H,8H-dihydro-[1,2,4] triazolo [1,5-α] pyridine - 6- carbonitril on colitis rats. Acetic acid induced colitis in rats is one of the common models in IBD research and resembles human ulcerative colitis in histology.[19,20,21,22,23] To test our hypothesis, the present study was undertaken to determine the possible mechanism of action of TRP 1 on the acetic acid induced ulcerative colitis in wistar rats.

MATERIALS AND METHODS

Materials

Adult male Wistar rats (200 - 220 g) were purchased from Mahaveer enterprises, Hyderabad. The animal room was maintained at 22°C–24°C and a lighting regimen of 12 hr light/12 hr dark. Rats were fed with standard house chow and water *ad libitum*. All animal experiments were performed after getting prior approval from the Institutional Animal Ethics Committee. TRP 1 procured from Chemistry department (Shri Vishnu college of Pharmacy), acetic acid (Loba Chemie), NBT- (Loba Chemie), reduced glutathione (Otto Chemie), trichloro acetic acid (Loba Chemie). ethylenediamino tetra acetic acid (Loba Chemie). O-Dianisidine (Loba Chemie). 2,2 Dipheny picryl hydrazyl (Siaco Research laboratory Pvt.Ltd.), 5, 5 DithioBis 2 Nitro benzoic acid -Siaco Research laboratory Pvt.Ltd, TNF α, IL-1β and IL-6 (Ray Biotech inc.). [3H]CCPA ([3H]2-chloro-N6-cyclopentyladenosine) was obtained from NEN Life Sciences (48.6 Ci/mmol), [3H]MSX-2([3H]3-(3-hydroxypropyl)-7-methyl-8-(m-methoxystyryl)-1-propargylxanthine) from Amersham (85 Ci/mmol), [3H]PSB-603 (8-(4-(4-(4-chlorophenyl)piperazine-1-sulfonyl)phenyl)-1-propylxanthine) from GE Healthcare (73 Ci/mmol), and [3H]PSB-11 ([3H]-8-ethyl-4-methyl-2-phenyl-(8R)-4,5,7,8-tetrahydro-1H-imidazo[2,1-i]-purin-5-one) from Quotient Biosciences (53 Ci/mmol). All other chemicals used were of analytical grade.

Radioligand binding studies

The binding studies were conducted at Human A_{2B} following the reported procedure.[24] In brief, membranes for radioligand binding were prepared from CHO cells stably transfected with human Adenosine receptors subtypes in a two-step procedure. In a first low-speed step (1000 g), cell fragments and were removed. The crude membrane fraction was sedimented from the supernatant at 100000 g. The membrane pellet was resuspended in the buffer used for the respective binding experiments, frozen in liquid nitrogen and stored at -80°C. For the measurement of adenylyl cyclase activity, only one high speed centrifugation of the homogenate was used. The resulting crude membrane pellet was resuspended in 50 mM Tris/HCl, pH 7.4 and immediately used for the cyclase assay.

Adenylyl Cyclase Activity

The potency of antagonists at the $A_{2B}R$ was determined in adenylyl cyclase experiments. The procedure was carried out as described previously with minor modifications. Membranes were incubated with about 150000 cpm of ATP for 20 min in the incubation mixture as described without EGTA and NaCl. For agonists, the EC_{50} values for the stimulation of adenylyl cyclase were calculated with the Hill equation. Hill coefficients in all experiments were near unity. IC_{50} value for concentration-dependent inhibition of NECA-stimulated adenylyl cyclase caused by antagonists was calculated accordingly. Dissociation constants (ki) for antagonists were calculated with the Cheng and Prusoff equation.

Antioxidant activity by *in vitro*

Antioxidant activity was tested by scavenging of DPPH* assay,[25] NO* assay,[26] SO* assay,[27] Fe^{+2} ascorbate induced lipid peroxidation assay.[28]

Anti-inflammatory activity by human RBC (HRBC) method[29]

Blood was collected from the healthy volunteers and mixed with equal volume of sterilized Alsevers solution (composition Glucose 20.5 g, Sodium chloride 4.2, Tri-sodium citrate 8.0 g, and citric acid 0.55 g, distilled water 1000 mL). Blood solution was centrifuged at 3000 rpm and the packed cells were separated, then washed with isosaline (0.85%; P^H 7.2) solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory property.

Different concentrations of TRP1(100 ng/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml), standard Sulfasalazine and control were separately mixed with 1ml of phosphate buffer (0.15 M, pH 7.4), 2mL of hyposaline (0.36%) and 0.5mL of HRBC suspension. All the assay mixtures were incubated at 37°c for 30 min and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuring the hemolysis produced in the control as 100%. Instead of hyposaline 2 mL of distilled water was employed as control. The anti-inflammatory potency was estimated by measuring % of inhibition of hemolysis

$$\text{Percentage inhibition of Hemolysis} = [1- (ABS_{sample} / ABS_{control})] \times 100.$$

Acute toxicity

The acute oral toxicity was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental procedure for the study the protective effect of TRP 1 on acetic acid induced ulcerative colitis

Animals were divided into five groups (n=6). In this study Sulfasalazine used as standard compound due to their potential anti-inflammatory activity against ulcerative colitis in rats and also used clinically.[30,31]

Group I: Serve as sham control

Group II: Animals were pretreated with 80% DMSO for 14th days and 2 ml of 3.0% acetic acid administered intra rectally on 14th day

Group III: Animals were pretreated with standard Sulfasalazine (360 mg/kg bd.wt.) for 14 days + 2 ml of 3.0% acetic acid administered intra rectally on 14th day.

Groups IV: Animals were pretreated with TRP 1 (1mg/kg bd.wt. dissolved in 80% DMSO) for 14 days + 2 ml of 3.0% acetic acid administered intrarectally on 14th day.

Groups V: Animals were pretreated with TRP 1 (10 mg/kg bd.wt.in 80% DMSO) for 14 day + 2 ml of 3.0% acetic acid administered intra rectally on 14th day.

Assessments of colitis

Animals were scarified at the end of treatment, the distal 10 cm portions of the colon were removed and cut longitudinally, cleaned with physiological saline to remove fecal residues.

Macroscopic inflammation scores are assigned based on the clinical features of the colon using an arbitrary scale ranging from 0 to10 as follows:

0 = No damage,
1 = Focal hyperemia (water oozes out),
2 = Ulcerization without hyperemia or bowel wall thickness,
3 = Ulcerization with inflammation at one site,
4 = Ulcerization with inflammation at two sites,
5 = Major sites of inflammation >1 cm along the organ with redness,
6 = Major sites of inflammation >2 cm along the organ with redness,
7 = Major sites of inflammation >3 cm along the organ with redness,
8 = Major sites of inflammation > 4 cm along the organ with redness,
9 = Major sites of inflammation >5 cm along the organ with redness and bleeding, and
10 = Major sites of inflammation >6 cm along the organ with redness, swelling, and bleeding.[32]

Biochemical assays

The colorectal tissue was collected, homogenized in 10 mM Tris-HCl buffer (p^H7.1). The homogenate was used for the measurement of antioxidant enzyme levels such as catalase,[33] glutathione,[34] colonic MPO activity,[35] inflammatory cytokines such as TNF-α, IL-1β and IL-6 (Ray Biotech Inc., US) using standard sandwich enzyme-linked immune sorbent assay (ELISA) kit specific for rat cytokines according to the manufacturer's instruction.

Histolopathological study

Table 1: Effect on human adenosine receptors (hA_{2b})

S. No	Compound	hA_{2B} ($k_1$nm)
1	TRP 1	30,000

Table 2: Effect on hemolysis

S. No	Sample	µg/ml				
		0.1	1	10	100	1000
1	Sulfasalazine	11.2±1.3	13.1±1.2	20.3±1.45	32.3±2.3	39.8±3.1
2	TRP 1	23.06±2.3	36.94±2.1	46.39±1.8	54.79±1.2	60.09±3.2

Table 3: Effect on free radical scavenging activity

S. No	Concentrations (µg/ml)	DPPH* free radicals (% of Inhibition)	NO* free radicals (% of Inhibition)	SO* free radical (% of Inhibition)	lipid peroxidation activity (% of Inhibition)
1	0.1	1.72±0.014	1.82±0.014	6.76±0.066	3.29±0.09
2	1	3.15±0.011	16.46±0.08	11.86±0.2	24.35±0.08
3	10	6.44±0.026	26.14±0.08	20.33±0.011	30.37±0.08
4	100	27.17±0.011	34.36±0.06	30.43±0.12	49.36±0.06
5	1000	28.07±0.011	44.03±0.05	35.36±0.03	58.22±0.08

A portion (2 cm) of the colonic specimen from each rat (n= 6) was fixed in 10% formalin, cut into 5 µm thickness, stained using heamatoxylin–eosin and histopathological observations were made. The stained sections of colon were examined for any inflammatory changes like infiltration of the cells, necrotic foci and damage to tissue structures like payers patches, damage to nucleus.

In silico method

To evaluate the compound TRP 1 binding capacity by using AUTODOCK 4.2 version and the images are rendered using Accelry's Discovery studio vizualizer v4.0 interface.

Statistical analysis

All data values are expressed as mean ± SD. Statistical analyses were performed using a one-way analysis of variance (ANOVA) followed by Dunnett's test, using (Graph pad version 5.0) *P<0.05, **P<0.01, ***P<0.001 were considered as statistically significant.

RESULTS

Effect on human adenosine receptors

Compound TRP 1 exhibited inhibitory concentration toward A_{2B} receptors is 30,000 nM (Table 1).

Effect on hemolysis

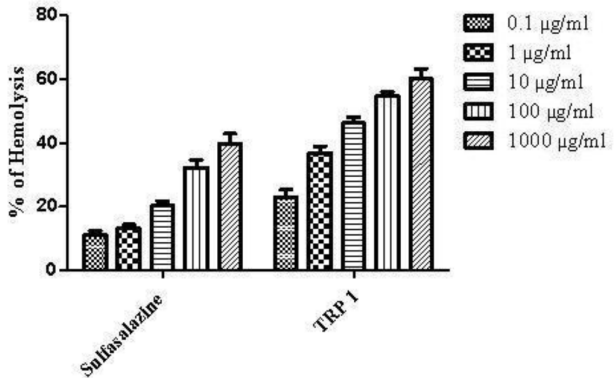


Figure 1: Effect on hemolysis.

At the dose of 1000 µg/ml of TRP 1 exhibited 60.09±3.2% protection of HRBC in hypotonic solution (Table 2, Figure 1).

Effect on free radical scavenging activity

At the dose of 1 mg/ml of TRP 1 neutralize free radicals of DPPH (28.07±0.1%), SO (35.3±0.03%), NO (44.03±0.05%) and LPO (58±0.8%) all the results compared with Sulfasalazine (Table 3, Figure 2).

Acute toxicity

TRP 1 treated rats were safe upto the dose level 2000 mg/kg bd.wt. As per OECD guidance 423. At the dose level of 300 mg/kg bd.wt. And 2000 mg/kg bd.wt. Treated rats were exhibited drowsy.

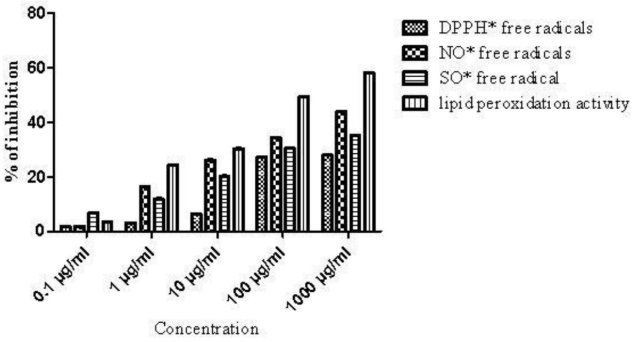


Figure 2: Effect on free radicals scavenging activity.

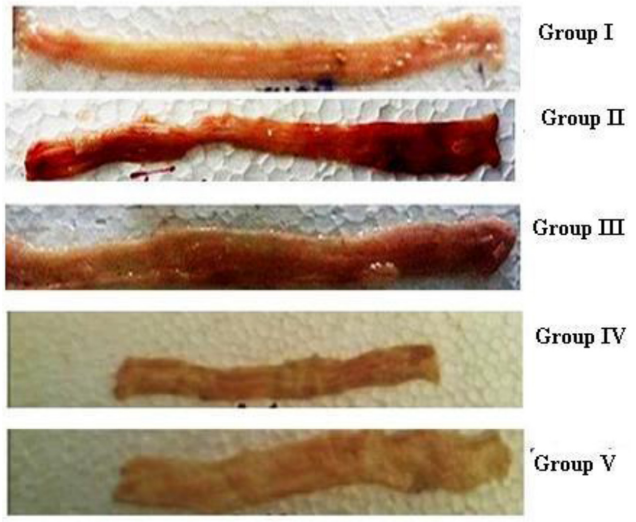


Figure 3: Effect on colon macroscopic score in colitis rats.

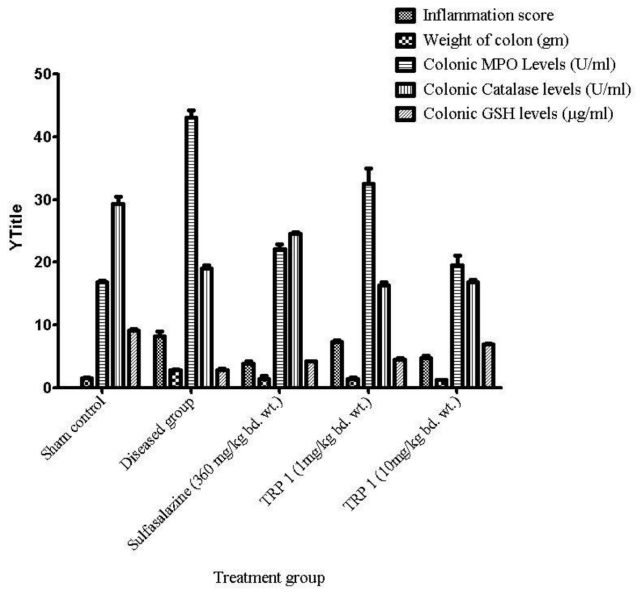


Figure 4: Effect on colon parameters in acetic acid induced colitis rats.

Table 4: Binding energy and inhibition of IL1-beta, IL6 and TNF-α by *in silico*

S. No	Drug targeting Protein	Binding Energy in Kcal/mol	IC_{50} (mM)
1	IL 1β	-6.7	7.5
2	IL 6	-6.2	28.65
3	TNF α	-5.92	45.87

Effect on colon parameters in acetic acid induced colitis rats

At the end of the treatment, acetic acid administered rats exhibited severe macroscopic edematous inflammation in the colon. The inflammation score and weight of colon was significantly (***P<0.001) increased in colitis rats 8.3±0.7, 2.9±0.3 respectively, also increase in content of MPO (43±1.28, ***P<0.001), decrease colonic catalase (19±0.31, ***P<0.001) and glutathione (2.9 ± 0.16) when compared to normal rats. The pretreatment of TRP 1 (10 mg/kg bd.wt.) significantly reduces inflammation score (4.83 ±0.3; **P<0.01), colon weight (1.25± 0.03; **P<0.01), colonic MPO activity (19.6±1.5; *P<0.05) and increases catalase activity (16.8 ± 0.38; *P<0.05), colonic GSH (6.9 ± 0.20; **P<0.01), the alteration in these biochemicals parameters when compared to colitis rats (Figure 3 and 4; Table 5).

Effect on cytokines TNF-α, IL-1β and IL-6 levels

Pro inflammatory cytokines are in acetic acid induced colitis rats showed significantly increased TNF-α (3800 ± 54.9; **P<0.01), IL 1β (5200 ±73.1; **P<0.01) and IL-6 (700 ± 90.4; **P<0.01) compared with those in the sham control group. TRP1 at the doses 1 mg/kg bd.wt. and 10 mg/kg bd. Wt., decreases the level of TNF-α from 3800 ± 54.9 pg/g tissue to 3200±22.8 pg/g, 3800 ± 54.9 pg/g tissue to 2900±22.6 pg/g (*P<0.05), respectively; correspondingly decrease of IL-1β from 5200±73.1 pg/g tissue to 4300±28.8 pg/g, 5200±73.1 pg/g tissue pg/mg to 3900±33.9 pg/mg tissue (**P<0.01), respectively. Correspondingly decrease of IL-6 from 700±90.4 pg/g tissue to 650±13.1 pg/g, 700±90.4 pg/g tissue pg/mg to 400±18.7 pg/mg tissue (**P<0.01), respectively (Table 5, Figure 5).

Binding energy and inhibition of IL1-beta, IL6 and TNF-α by *in silico*

Docking is widely used in modern drug discovery process and effective tool for quickly and accurately predicting biomolecular conformation with binding energy of protein ligand complex. Compound TRP 1 exhibited potent inhibition on IL-1β (-6.7 Kcal/mol, 7.5 mM), TNF α (-5.92 Kcal/mol, 45.87 mM) and IL 6 (-6.2 Kcal/mol, 28.65 mM) (Table 4).

Table 5: Effect on colon parameters in acetic acid induced colitis rats

S. No	Treatment group	Inflammation score	Weight of colon (gm)	Colonic MPO Levels (U/ml)	Colonic Catalase levels (U/ml)	Colonic GSH levels (µg/ml)	TNF-α (pg/g tissue)	IL-1β(pg/g tissue)	IL-6(pg/g tissue)
1	Sham control	0±0	1.6±0.11	16.9±0.24	29.3±1.14	9.2±0.17	1200 ± 29.8	2300 ±43.3	190 ± 11.9
2	Diseased control	8.3±0.70***a	2.9±0.13***a	43±1.25***a	19.1±0.51***a	2.9±0.16***a	3800 ± 54.9 **a	5200 73.1***a	700 ±90.4***a
3	Sulfasalazine (360 mg/kg bd. wt.)	3.9±0.4*b	1.4±0.5*b	22.08±0.8*b	24.5±0.3*b	4.2±0.1*b	2900±25.2*b	4300±22.2*b	450±21.1*b
4	TRP 1 (1 mg/kg)	7.33±0.33	1.41±0.03	32.5 ± 2.5*b	16.3±0.49*b	4.5±0.29	3200 ± 22.8*b	4300 ± 28.8	650± 13.1
5	TRP 1 (10 mg/kg)	4.83±0.30**b	1.25±0.03**b	19.6±1.5*b	16.8±0.38*b	6.9±0.20**b	2900 ± 22.6*b	3900 ±33.9 **b	400 ±18.7**b

Data are expressed as Mean ± SD, from six groups of rats and analyze by one way ANOVA fallowed by Dennett's test.
*P< 0.05, **P < 0.1, ***P<0.001;
a compare with sham control, b compared with disease control.

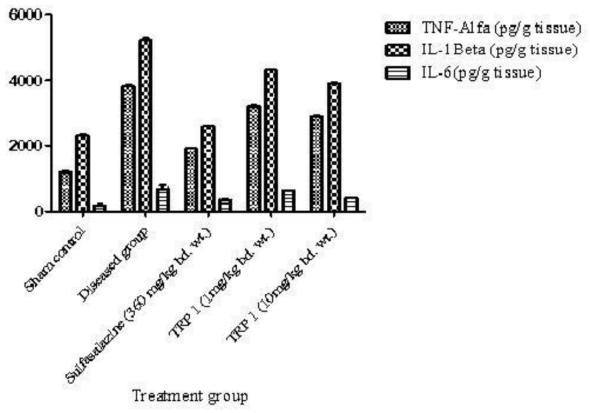
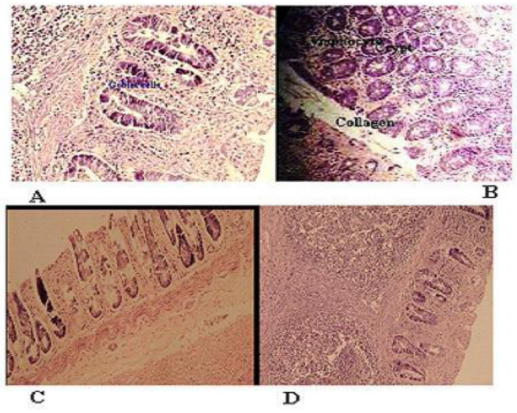


Figure 5: Effect on cytokines TNF-α, IL-1β and IL-6 levels.



A. Normal intact mucosa from normal control and intact epithelial surface, Goblet cell B. Acetic acid induced colitis showing massive necrotic destruction of epithelium, submucosal edema, areas of haemorrhages and Lymphocyte cell, Collagen C Acetic acid+ TRP 1 (1 mg/kg bd.wt.) showing minimal damage of the mucosa with slight submucosal edema and mild inflammatory cell infiltration. D. Acetic acid+ TRP 1(10 mg/kg bd.wt.) showing significant protection of colonic mucosa from acetic acid induced colitis damage.

Figure 6: Histopathological changes in colon of experimental rats.

Histopathological changes in colon of experimental rats

Acetic acid induced colitis showed massive necrotic destruction of epithelium, submucosal edema, areas of haemorrhages and inflammatory cellular infiltration. TRP 1 at low dose level showed minimal damage of the mucosa with slight submucosal edema and mild inflammatory cell infiltration. 10 mg/kg bd.wt. TRP 1 showed remarkable recovery of colonic mucosa from acetic acid induced colitis damage (Figure 6).

DISCUSSION

Compound TRP 1 has been demonstrated to have protective effect against acetic acid induced ulcerative colitis. Adenosine plays prominent roles in maintaining tissue integrity by modulation of immune functions, down-

regulation of phlogistic reactions, interference with the biosynthesis of proinflammatory cytokines and inhibition of neutrophil adhesion, degranulation and antioxidant activity.[36] A number of studies are reported that the adenosine agonists are trigger proinflammatory responses and neutrophile infiltration through stimulation of Adenosine receptors on neutrophils and lymphocytes.[37,38,39] Previous studies are revealed that upregulation of A_{2B} receptors are contributing in colitis pathology[40] which was attenuated by Adenosine receptor antagonists.[41,42] In present study demonstrated that compound TRP 1 antagonize the A_{2B} receptors proved in radioligand binding studies and also exhibited anti-inflammatory activity by *in vitro*. Oxidative stress plays an important role in pathophysiology of ulcerative colitis and there is direct evidence that generation of reactive oxygen species attack the cellular macromolecules, disrupt epithelial cell integrity.[43,44] Acetic acid exerts damaging effect by an acute inflammatory response following colonic injury, accompanied by widespread hemorrhage, release of mediators, and formation of lesions. The protonated form of the acid liberates protons within the intracellular space, causing a massive intracellular acidification resulting in an immense epithelial damage.[45] The weight of the inflamed colonic tissue is considered as a reliable and sensitive indicator for the severity, extent of intestinal inflammation, over production of TNF α, IL-1β and IL-6 on intrarectal administration of acetic acid.[46,47,48,49] Consistent with this notion, the results of this study revealed, acetic acid treated rats was showed significant increase colon weight and macroscopic damage score, indicative of formation of ulcers, edema and increases inflammatory cytokines levels such as TNF α, IL-1β and IL-6. Pretreatment with TRP 1 in acetic acid induced colitis significantly reduced the weight of inflamed colon weight, macroscopic damage compare with colitis rats and reverse elevated TNF α, IL-1β and IL-6 levels and prevent epithelial damage indicating its protective effect from ulcerative colitis. Indeed, the *in vitro* DPPH assay, Hydrogen peroxide scavenging assay, superoxide free radical scavenging assay, Fe^{2+} ascorbate induced lipid peroxidation assay was performed to evaluate antioxidant potential of TRP 1, results indicated that compound to be endowed with free radical inhibitory activity. *In silico* studies also concluded that TRP 1 showed potent inhibition on IL1β, IL6 and TNF-α proteins.

MPO is an enzyme present in neutrophil, the levels of MPO activity proportional to the neutrophil concentration inflamed tissue. Therefore measurement of MPO activity has been considered a sensitive assay for acute intestinal inflammation. In addition increased MPO activity has been reported to an index of neutrophil infiltration and inflammation[50] and also enzyme catalyzes the formation of potent cytotoxic oxidants such as hypochlorous acid from H_2O_2 and chloride ions.[51,52] MPO activity was increased by acute administration of acetic acid through rectal route[53] and its activity significantly reduces in TRP 1 treated colitis rats. Suppression of MPO activity by compound TRP 1 indicates inhibition of neutrophil infiltration in the colonic mucosa.

GSH and catalase plays a vital role in protecting tissues against damage by scavenging oxidant products. Earlier studies revealed that GSH level has been reduced in tissues when antioxidant was neutralized by liberated oxygen derived free radicals.[54] Several studies are revealed that intrarectal administration of acetic acid to decreases antioxidant defensive system such as GSH and catalase activity.[23] Similarly current scientific study was observed increase in GSH and catalase activity in the compound TRP 1 treated colitis groups. Hence the results suggest that the defense system was improved by the treatment of TRP 1.

CONCLUSION

TRP 1 exhibited antagonist on A_{2B} receptors, possess anti-inflammatory activity and reduces inflammatory mediator's levels, enhanced antioxidant activity in acetic acid induced colitis in rats. All the above scientific evidence suggested that the selected compounds improve clinical score in acetic acid induced colitis rats.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION USED

TRP: 1-(7-amino-5-oxo-2- phenyl)-5H,8H-dihydro-[1,2,4] triazolo[1,5-α] pyridine - 6- carbonitril),A 2B- Adenosine 2B; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **IBD:** Inflammatory bowel disease; **TNF:** Tumor necrosis factor alpha; **IL:** Interleukins.

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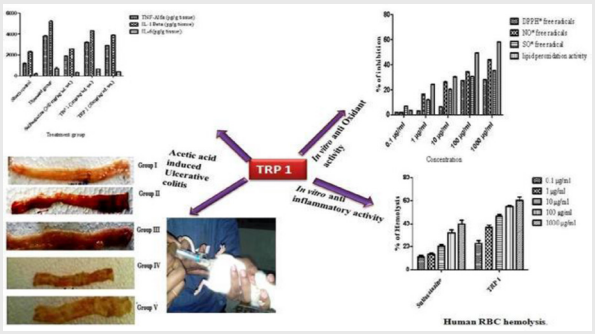
PICTORIAL ABSTRACT



SUMMARY

Ulcerative colitis is an inflammatory bowel disease that causes long lasting inflammation and sores (ulcers) in the innermost lining of large intestine (colon) and rectum.

Activation of A_{2B} receptors and depletion of antioxidant defensive system are contributing factors in development of ulcerative colitis.

TRP 1 antagonize the A_{2B} receptors, proved in radioligand binding assay and also exhibited anti-inflammatory activity, antioxidant activity reported in *in vitro* models.

Pretreatment with TRP 1 significantly minimizes the proinflammatory mediators release as well as enhance antioxidant defensive system in acetic acid induced ulcerative colitis rats.

Eventually TRP 1 evoked a significant protective effect against colonic damage induced by intrarectal injection of acetic acid.

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