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Original Article

# Protective Effect of A2B Receptor Antagonist (TRP 2) on Acetic Acid Induced Ulcerative Colitis in Rats: *In vitro, in vivo* and *in silico* Methods

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## Abstract

Present study was designed to elucidate the protective effect of pyridinone derivatives such as 7-amino-5-oxo- 2- (4-hydroxy-phenyl) -5H,8H-dihydro-[1,2,4] triazolo [1,5-α] pyridine - 6- carbonitril (TRP 2) against acetic acid induced ulcerative colitis in rats by *In vitro, in vivo and in silico* methods. *In vitro*, Radioligand binding assay on human adenosine receptors (A2B) revealed that at a concentration of 3000nm these receptors were antagonized. TRP 2 significantly neutralizes the free radicals of DPPH, SO, NO and LPO at $IC_{50}$ 300 µg/ml, 100 µg/ml, 50 µg/ml and 5 µg/ml respectively. *In vivo,* Intra rectal administration of acetic acid caused a significant increase on macroscopic score, colon weight, colonic MPO, TNF-α, IL 6 and IL 1β levels, while TRP 2 treated colitis rats has exhibited improved glutathione and catalase antioxidant activity, furthermore TRP 2 exhibited inhibitory action on TNF α, IL 1β, IL 6 and the myeloperoxidase activity. *In silico*, $IC_{50}$ of TRP 2 against IL 1β, IL 6 and TNF-α were 3.04 mM, 39.21 mM and 48.35 mM respectively. TRP 2 treatment improved clinical score in acetic acid induced colitis in rats by reducing the inflammatory mediators and subsequent improvement of antioxidant activity in colitis rats through A2B receptor antagonist property.

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## Introduction

IBD, including Crohn's disease (CD) and ulcerative colitis (UC), is a lifelong disabling gastrointestinal disease (1). 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 (2) and imbalances between proinflammatory cytokines, such as 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 (2). 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, which are expressed on the surface of immune cells. $A_{2B}$ R are highly expressed in the cecum and colon, esophagus, stomach, and jejunum but appears to be absent in the ileum (3). 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 (4, 5, 6). Past scientific studies supported that fused pyridinone ring derivatives were found to be a versatile pharmacophore with wide range of useful biological activities due to adenosine receptors antagonizing property (7, 8, 9) and the ameliorated inflammatory rate (10). Hence $A2_B$ R are great deal of interest, its primary molecular target and its mechanism of action remain to be clarified.

The present study evaluated the protective effect of pyridinone derivatives like 7-amino-5-oxo- 2- (4-hydroxy-phenyl) -5H,8H-dihydro-[1,2,4] triazolo [1,5-α] pyridine - 6- carbonitril on colitis rats. Currently, few of the experimental animal models are used to study the pathogenesis and pathophysiology of the inflammatory bowel disease. Acetic acid induced colitis in rats is one of the common models in IBD research and resembles human ulcerative colitis in histology (11, 12, 13, 14). to test our hypothesis, the present study was undertaken to determine the possible mechanism of action of TRP 2 on the acetic acid induced ulcerative colitis in Wistar rats.

## Materials and Methods

### Materials

Adult male Wistar rats (200-250 g) were purchased from Mahaveer enterprises, Hyderabad. The animal room was maintained at 22°C–24°C and a lighting regimen of 12 hour light/12 hour 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 (439/PO/01/a/CPCSEA). TRP 2 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),. All other chemicals used were of analytical grade.

### *In vitro* Studies

#### Radioligand binding studies

Binding at human $A_1$, $A_{2A}$ and $A_3$ ARs Binding studies at $hA_1$, $hA_{2A}$, and $hA_3$ ARs were carried out by method of Klotz KN *et al.,* 1997 (15). Chinese hamster ovary (CHO) cells stably transfected with human (h) $A_1$, $A_{2A}$, and $A_3$ ARs subtypes were used for the preparation of membranes for radioligand binding studies, 10 nM [3H]-5'-N-ethylcarboxamido adenosine ([3H] NECA) used as radioligands for $hA_{2B}$. Nonspecific binding of [3H]CCPA was determined in presence of 1 mM theophylline, while 100 µM (R)-N6 -phenyliso-propyladenosine (R-PIA) was used for [3H]NECA. Calculation of Ki values from competition experiments was carried out by using the program SCIFIT (38).

$$K_i = IC_{50} (1+A^k/K_p)$$

$IC_{50}$ Is the concentration of antagonist producing 50% inhibition; A is the concentration of agonist against which the $IC_{50}$ is being determined; $K_p$ is the apparent equilibrium constant of the agonist.

**Adenylyl cyclase activity:**

Adenylyl cyclase activity due to the lack of a high affinity radioligand for $A_{2B}$ AR adenylyl cyclase experiments were carried out as a measure of affinity for $hA_{2B}$ AR by model of Cheng HC 2001 (38). Membranes were prepared from CHO cells stably transfected with $hA_{2B}$ ARs followed by incubation with 100 nM NECA as well as 150,000 cpm of [$\alpha$-32P] ATP. All the target compounds were tested at different concentration for 20 min in the incubation mixture without using EGTA (ethylene glycoltetraacetic acid) and NaCl. None of the compounds showed measureable interaction with the $hA_{2B}$ AR (IC50 values > 90 µM; data not shown).

**Antioxidant activity**

Antioxidant activity was tested by scavenging of DPPH* assay by method of Blois MS 1958 (16), NO* assay by method of Sreejayan N *et al.,* 1997 (17), SO* assay by method of Liu F *et al.,* 1997 (18), $Fe^{+2}$ ascorbate induced lipid peroxidation assay by method of Ohkawa H *et al.,* 1997(19).

**Estimation *in* anti inflammatory activity using human red blood cells (HRBC) method of Azeem AK *et al.,* 2010 (20)**

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, 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%; PH 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 TRP-2 (100 ng/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml), standard Sulfasalazine and control were separately mixed with 1 ml of phosphate buffer (0.15 M, pH 7.4), 2 mL of hyposaline (0.36%) and 0.5 mL 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

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

**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.

***In vivo* studies**

**Study the protective effect of TRP 2 on acetic acid induced ulcerative colitis by method of Rashidian A et al., 2014 (21).**

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 (22).

Group I: Serve as sham control

Group II: Rats were pretreated with DMSO for 14th days and 2 ml of 3.0% acetic acid administered intra rectally on $14^{th}$ day

Group III: Rats were pretreated with Sulfasalazine (360 mg/kg bd.wt. p.o.) for 14 days and 2 ml of 3.0% acetic acid administered intra rectally on $14^{th}$ day.

Groups IV Rats were pretreated with TRP 2 (1 mg/kg bd.wt. p.o) for 14 days and 2 ml of 3.0% acetic acid administered intrarectally on $14^{th}$ day.

Groups V Rats were pretreated with TRP 2 (10 mg/kg bd.wt.p.o) for 14 days and 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 >6cm along the organ with redness, swelling, and bleeding (23).

#### Biochemical assays

The colorectal tissue was collected, homogenized in 10 mM Tris-HCl buffer (pH7.1). The homogenate was used for the measurement of antioxidant enzyme levels such as catalase (24), glutathione (25), colonic MPO activity (26), inflammatory cytokines such as TNF-$\alpha$, IL-1$\beta$ 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.

#### Histopathological assessment of colitis

Colonic specimens were fixed in 10% formalin in phosphate buffered saline, embedded in paraffin, after several steps to induce dehydration in alcohol, sections of 4-ìm thickness were prepared and stained with hematoxylin and eosin (H&E). Thereafter, histopathological analysis was carried out using a EVOS-xl CORE light microscope (AMG, Bothell,WA). All samples were analyzed in a blinded manner. A certified histopathologist performed all analyses/ interpreted the observed outcomes.

#### *In silico* method

To evaluate the compound TRP 2 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 by radio ligand binding studies

Table I shows that the compound TRP 2 exhibited

TABLE I : Activity of the compound TRP 2 on radioligand binding affinity towards human adenosine receptors.

| *S. No.* | *Compound* | *hA2B($k_i$nm)* |
|---|---|---|
| 1 | TRP 2 | 30,000 |

inhibitory concentration toward A2B receptors at 30 nM.

**Effect on human RBC lysis using HRBC membrane stabilization.**

Table II, Fig. 1 shows that at the dose of 1000 µg/ml of TRP 2 exhibited 42.3±1.3% protection of HRBC in hypotonic solution. All results were compared with Sulfasalazine which showed 49.8±2% protection.

**Effect on free radical scavenging activity:**

Table III, Fig. 2 showed that, TRP 2 exhibited $EC_{50}$ for DPPH* (300 µg/ml), NO* (100 µg/ml), SO* (50 µg/ml) and LPO (5 µg/ml).

**Acute toxicity:**

TRP 2 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. treatnent exhibited sedation.

**Effect on colon parameters.**

Table IV, Figs. 3, 7 shows that the end of the treatment, acetic acid administered rats exhibited severe macroscopic edematous inflammation in the colon. The inflammation score and weight of colon were significantly (***P<0.001) increased in colitis rats 8.3±0.7, 2.9±0.3. Pre treatment of TRP 2 at the dose (10 mg/kg bd.wt.) showed significant decrease in (*P<0.05) in inflammation score and weight of colon. Intrarectal administration of acetic acid treated rats showed significant increase in content of MPO (43±1.28, **P <0.01) and decrease colonic catalase (19± 0.31, **P<0.01) when compared to normal rats. At the dose of 10 mg/kg bd.wt. TRP 2 significantly (*P<0.05) reduces the alteration in these

TABLE II : *In vitro* anti inflammatory activity of TRP 2 by using HRBC membrane stabilization method.

| *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 2 | 9.3±0.32 | 14.3±0.76 | 16.8±0.34 | 20.65±0.76 | 42.3±1.3 |

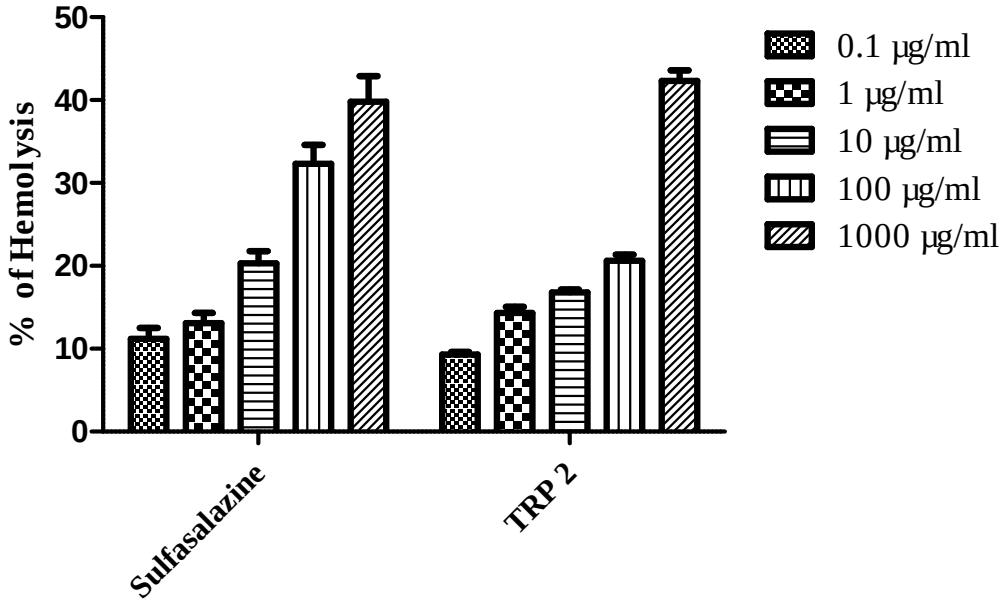


Fig. 1 : *In vitro* anti inflammatory activity of TRP 2 by using HRBC membrane stabilization method.

TABLE III: Effect of TRP 2 on free radicals 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 | 24.5±1.7 | 15±0.5 | 35±0.21 | 27±2.0 |
| 2 | 1 | 32±1.04 | 25±1.32 | 40±1.0 | 46±1.32 |
| 3 | 10 | 45.6±1 | 45±1.52 | 45±1.20 | 51±1.25 |
| 4 | 100 | 47±0.5 | 50±2.5 | 55±1.52 | 57±1.92 |
| 5 | 1000 | 52±1.5 | 62±1.2 | 60±1.32 | 63±2.08 |
| | $IC_{50}$ | 300 µg/ml | 100 µg/ml | 50 µg/ml | 5 µg/ml |

TABLE IV: Effect of TRP 2 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) |
|---|---|---|---|---|---|---|
| 1 | Sham control | 0±0 | 1.6±0.11 | 16.9±0.24 | 29.3±1.14 | 9.2±0.17 |
| 2 | Diseased group | 8.3±0.70***a | 2.9±0.13***a | 43±1.25***a | 19.1±0.51***a | 2.9±0.16***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 |
| 4 | TRP 2 (1 mg/kg bd. wt.) | 6.3±0.52 | 2.4±0.08 | 35.9±0.80 | 22±0.55 | 4.1±0.13 |
| 5 | TRP 2 (10 mg/kg bd. wt.) | 4.3±0.57**b | 2.1±0.07**b | 25.7±0.88**b | 24.5±0.85**b | 5.4±0.08**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 group.

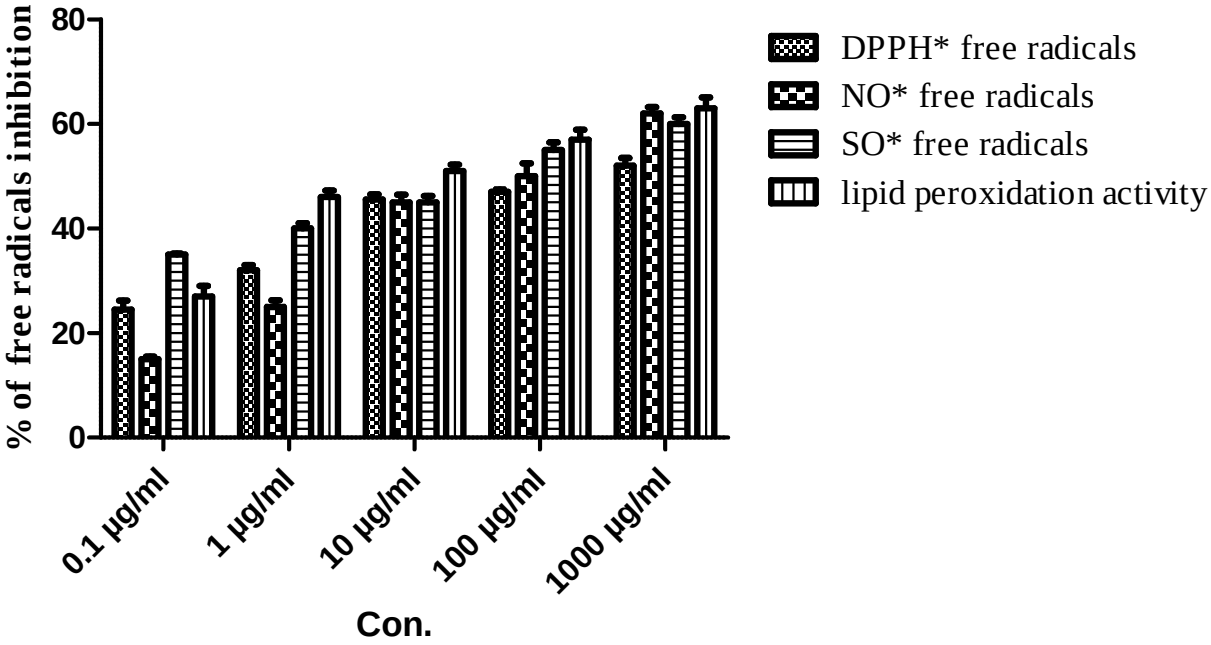


Fig. 2: Effect of TRP 2 on free radicals scavenging activity.

biochemicals parameters when compared to colitis rats.

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

Pro inflammatory cytokines TNF-α, IL 1β and IL-6 levels were significantly increased in acetic acid treated rats compared with those in the sham control group (***P<0.001). TRP 2 treated rats significantly reduced increased TNF-α, IL 1β and IL-6 in colitis rats. TRP 2 (1 and 10 mg/kg) decreases the level of TNF-α from 3800±54.9 pg/g tissue to 2700±42.8

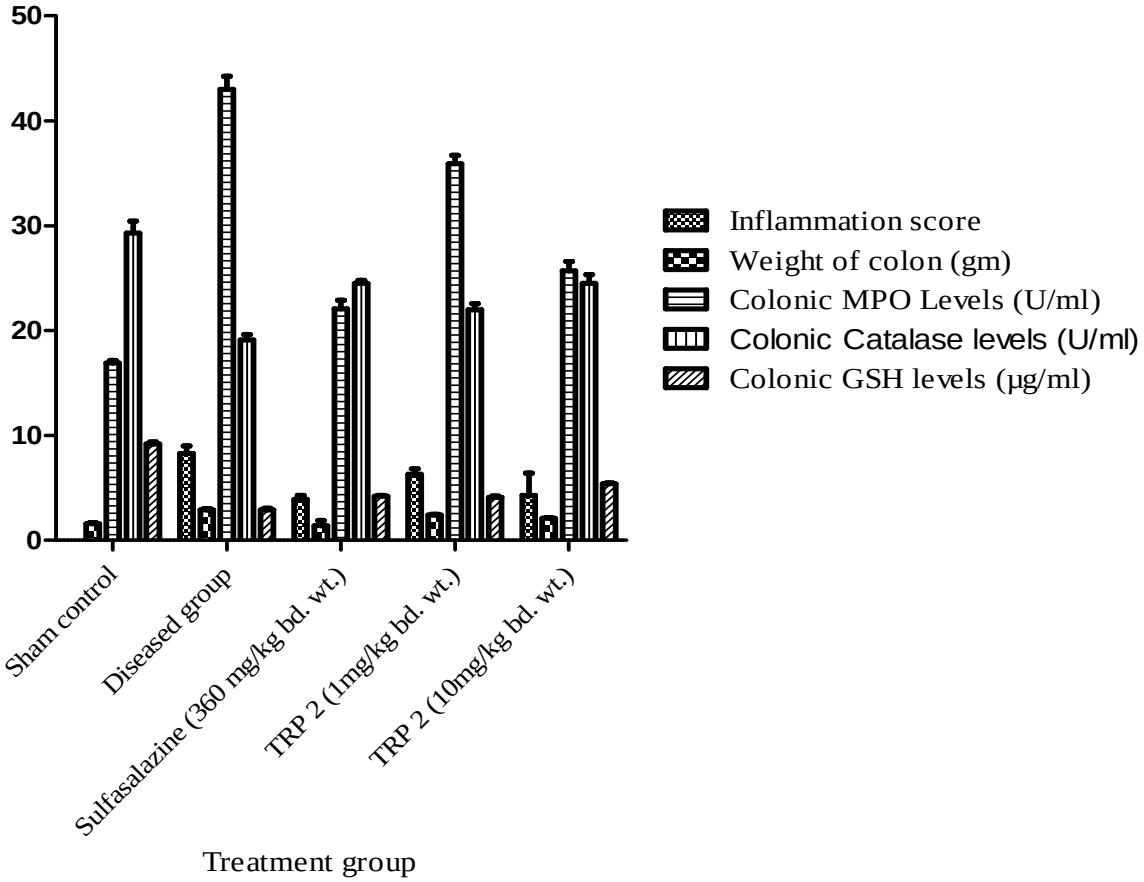


Fig. 3: Effect on colon parameters.

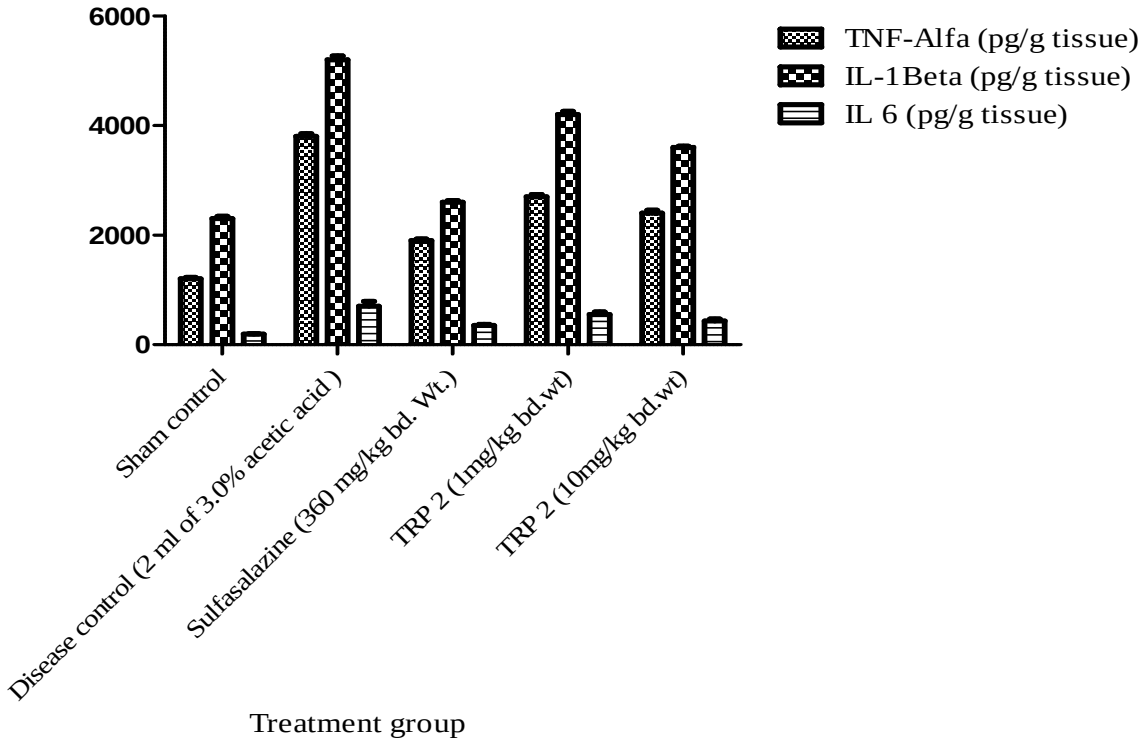


Fig. 4: Effect of TRP 2 on cytokines TNF-α, IL-1β and IL-6 levels in acetic acid induced colitis rats.

TABLE V: Effect of TRP 2 on cytokines TNF-α, IL-1β and IL-6 levels in acetic acid induced colitis rats.

| S.No | Treatment | TNF-α (pg/g tissue) | IL-1β (pg/g tissue) | IL 6 (pg/g tissue) |
|---|---|---|---|---|
| 1 | Sham control | 1200±29.8 | 2300±43.3 | 190±11.9 |
| 2 | Disease control (2 ml of 3.0% acetic acid ) | 3800±54.9***a | 5200±73.1***a | 700±90.4***a |
| 3 | Sulfasalazine (360 mg/kg bd. Wt.) | 1900±26.3*b | 2600±32.4*b | 350±23.8*b |
| 5 | TRP 2 (1 mg/kg bd.wt) | 2700±42.8*b | 4200±58.8*b | 550±49.6*b |
| 6 | TRP 2 (10 mg/kg bd.wt) | 2400±52.6**b | 3600±23.9**b | 430±38.7*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<0.01, ***P<0.001; a compare with sham control, b compared with disease group.

pg/g, 3800±54.9 pg/g tissue to 2400±52.6 pg/g (**P<0.01), respectively; correspondingly decrease of IL-1β from 5200±73.1 pg/g tissue to 4200±58.8 pg/g, 5200±73.1 pg/g tissue pg/mg to 3600±23.9 pg/mg tissue (**P<0.01), respectively. Study also signifies a comparable decrease of IL-6 from 700±90.4 pg/g tissue to 550±49.6 pg/g, 700±90.4 pg/g tissue pg/mg to 430±38.7 pg/mg tissue (*P<0.05), respectively (Table V Fig. 4).

**Histological assessment**

Histological examination of the colonic sections was also used to access the protective effect of TRP 2 on ulcerative colitis as presented in Fig. 5. Administration of acetic acid caused transmural necrosis in all layers of the bowel wall; infiltrate consisting of mixed inflammatory cells (mainly composed of lymphocytes and plasma cells) was

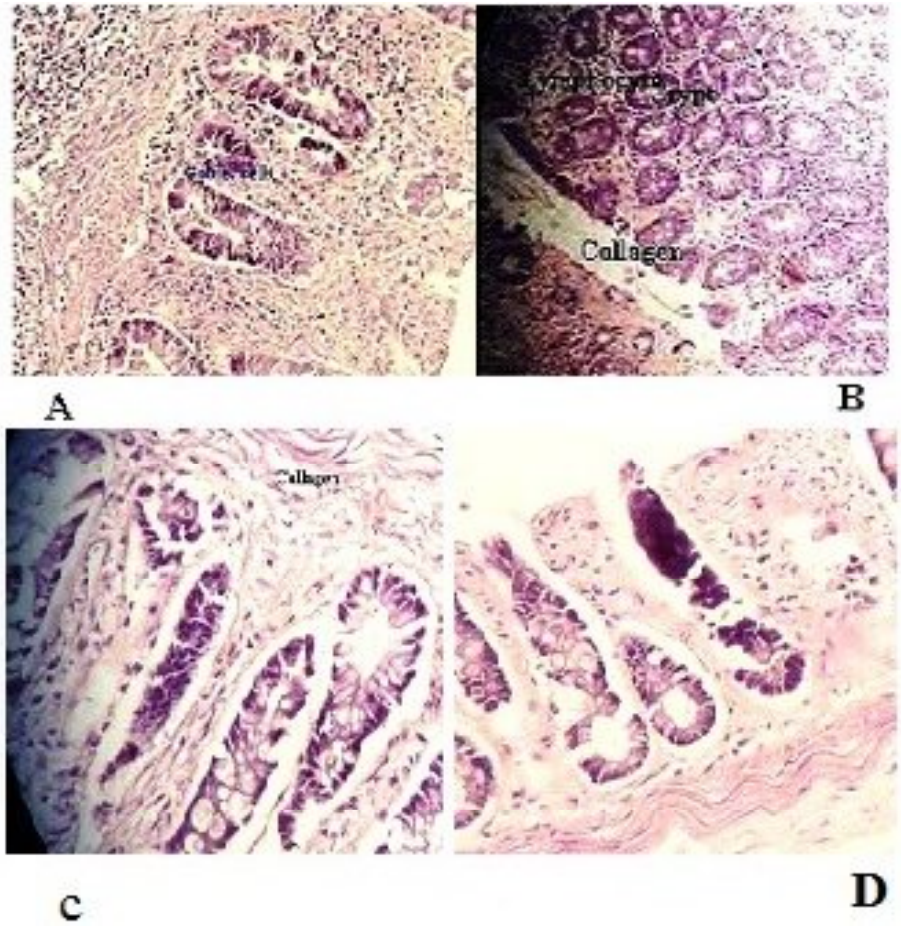


Fig. 5: Effect of TRP 2 on histological assessment.
A. Sham control B. Disease control C. TRP 2 (1 mg/kg bd. wt.) D. TRP 2 (10 mg/kg bd. wt.)

observed. Oral administration of TRP 2 treated animals significantly attenuated the histological signs of the cell damage, reduction in mucosal injury, edema and reduced infiltration of inflammatory cells lamina propria in comparison with the colitis group.

**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 2 exhibited potent inhibition on IL-1β (-7.53 Kcal/mol, 3.04 mM), TNF α (-5.89 Kcal/mol, 48.35 mM) and IL 6 (-6.01 Kcal/mol, 39.21 mM) (Table VI).

TABLE VI : Binding energy of TRP 2 and inhibition of IL1-beta, IL6 and TNF-α by in silico.

| *S. No.* | *Compound* | *Drug Target* | *Binding Energy in Ki cal/mol* | *Predicted $IC_{50}$ value in mM* |
|---|---|---|---|---|
| 1 | TRP 2 | IL1-Beta | –7.53 | 3.04 |
| 2 | | IL6 | –6.01 | 39.21 |
| 3 | | TNF-Alpha | –5.89 | 48.35 |

## Discussion

Compound TRP2 has demonstrated to have protective effect against acetic acid induced ulcerative colitis. Adenosine plays prominent role 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 anti-oxidant activity (27). Many studies reported that the adenosine agonists trigger proinflammatory responses and neutrophile infiltration through stimulation of Adenosine receptors on neutrophils and lymphocytes (28). Previous studies reveal that upregulation of A2B receptors, contributing in colitis pathology (4) which was attenuated by Adenosine receptor antagonists (29). Hence blocking of A2B receptor is a novel approach in the treatment of IBD. The present study demonstrated that compound TRP 2 antagonize the A2B receptors which is 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

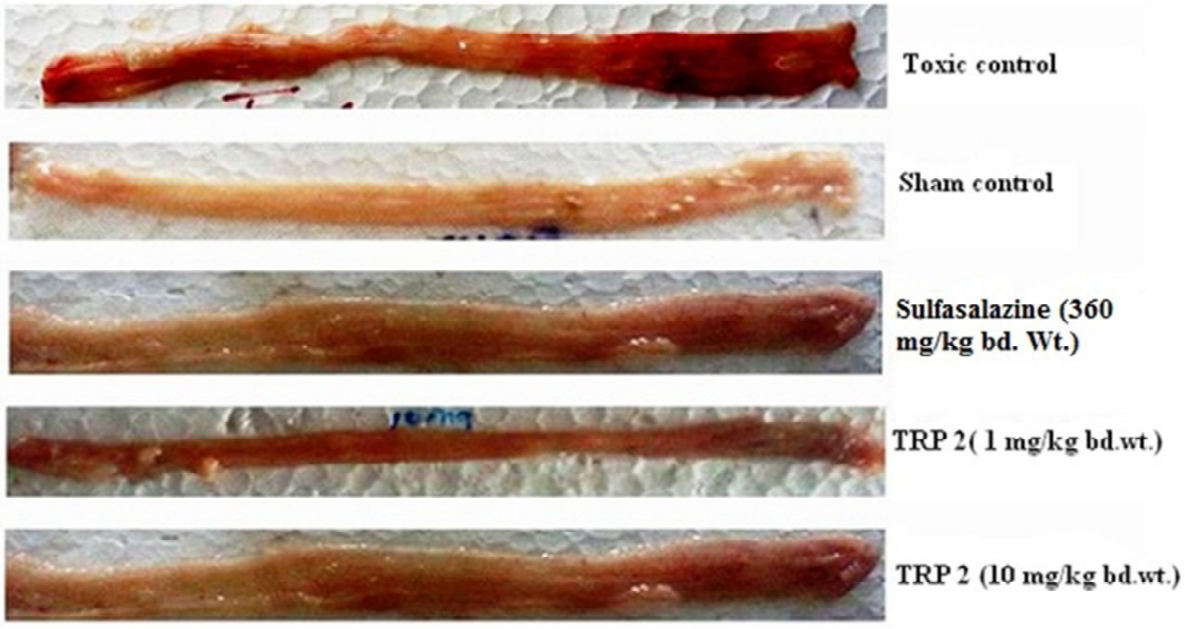


Fig. 6 : Colonic macroscopic studies.

integrity (30). 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 (22, 31). 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 $\alpha$, IL-1$\beta$ and IL-6 on intrarectal administration of acetic acid (32, 33). Consistent with this notion, the results reveal that, acetic acid treated rats has shown significant increase in colon weight and macroscopic damage score, indicative of formation of ulcers, edema and increased inflammatory cytokines levels such as TNF $\alpha$, IL-1$\beta$ and IL-6. Pretreatment with TRP 2 in acetic acid induced colitis significantly reduced the weight of inflamed colon weight, macroscopic damage compare with colitis rats and reverse elevated TNF $\alpha$, IL-1$\beta$ and IL-6 levels, 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 were performed to evaluate antioxidant potential of TRP 2, results indicated that compound to be endowed with free radical inhibitory activity. *In silico* studies also concluded that TRP 2 showed potent inhibition on IL1$\beta$, IL6 and TNF-$\alpha$ 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 as a sensitive assay for acute intestinal inflammation. In addition increased MPO activity has been reported as an index of neutrophil infiltration and inflammation (34) and also enzyme catalyzes the formation of potent cytotoxic oxidants such as hypochlorous acid from $H_2O_2$ and chloride ions (35). MPO activity was increased by acute administration of acetic acid through rectal route (36) and its activity significantly reduces in TRP 2 treated colitis rats. Suppression of MPO activity by compound TRP 2 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 (37). Several studies reveal that intrarectal administration of acetic acid decrease antioxidant defensive system such as GSH and catalase activity (14). Similarly current scientific study was observed to increase GSH and catalase activity in the compound TRP 2 treated colitis groups. Hence the results suggest that the defense system was improved by the treatment of TRP 2.

#### Conclusions

TRP 2 exhibited antagonist property on A2B receptors, possess anti inflammatory activity and reduces inflammatory mediator levels, enhanced antioxidant activity in acetic acid induced colitis in rats. All the above scientific evidence suggested that the TRP 2 improve clinical score in acetic acid induced colitis rats.

## Acknowledgements

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