Research Article
ISSN: 0974-6943

Darji V. C et al. / Journal of Pharmacy Research 2010, 3(12),2850-2852

Available online through
www.jpronline.info

Effect of *Punica granatum* fruits in inflammatory bowel disease.

Darji V.C [1*], Bariya A.H[2], Deshpande S.S[3], Patel D.A[4]

[1]*Department of Pharmacology, Sharda School of Pharmacy, Pethapur-Mahudi road, pethapur Gandhinagar-382610, Gujarat, India.*
[2]*Department of Pharmacology, Kalol Institute of Pharmacy, Opposite to Janpath hotel, Kalol-382721, Gujatat, India.*
[3]*Department of Pharmacology, K.B. Institute of Pharmaceutical Education & Research, Sector-23, Gandhinagar-382023, Gujarat, India.*
[4]*Department of Pharmacognosy, Arihant College of Pharmacy, S.G. Highway, Uavarsad, Gandhinagar-382422, Gujarat, India.*

Received on: 10-08-2010; Revised on: 18-10-2010; Accepted on:20-11-2010

ABSTRACT

It has been demonstrated that *Punica granatum* Linn. *(Punicaceae)* is useful as an adjunctive therapy for inflammatory bowel disease. However, its effect on ulcerative colitis has not been investigated. In the present study, *Punica granatum was* tested for 2, 4 - dinitro benzene sulfonic acid (DNBS) induced colitis, and antioxidant activity was evaluated to clarify its possible mode of action. Male albino wistar rats were randomly divided into five groups: Normal control (Group I), vehical contol (Group II), colitis induced by DNBS without any therapy (Group III), colitis treated with standard 5-Amino salicylic acid (Group IV), colitis treated with test drug *punica granatum* (Group V). Treatment was given for 18 days. Rats were sacrificed on the 18th day after the procedure. Malondialdehyde (MDA), Nitric oxide (NO), Myeloperoxidase (MPO), Superoxide dismutase (SOD), activity were measured in the isolated colon tissue. MDA, NO, MPO levels in colon tissue homogenate were decreased & SOD level was increased in group IV & Group V as compare to those of Group III. There was also increase in food intake, water intake, % body weight & decreased colon weight in Group IV & Group V as compared to Group III. There was also improvement in inflammatory indices of colon mucosal damage index (CMDI) & Disease activity index (DAI) & histopathology of Group IV & Group V as compared to those of group III. The results of our study suggest that *Punica granatum* therapy has beneficial effects on the course of experimental colitis.

Keywords: *Punica granatum* Linn. *(Punicaceae),* Inflammatory bowel disease (IBD), Antioxidant

INTRODUCTION

IBD is a chronic inflammatory disease of gastrointestinal tract. It comprises the two conditions, Crohn's disease and ulcerative colitis, characterized by chronic recurrent ulceration of the bowel. Although the exact etiopathogenesis of IBD is still not clear, it appears that there is chronic activation of the immunoinflammatory cascade with transient tethering of leukocytes to the endothelium[1]. This inflammatory response is most likely made possible by defects in both the mucosal immune system and the barrier function of the intestinal epithelium. Sequence of events involving control of infection, resolution of inflammation, differentiation & remodeling are the key processes for the treatment of IBD. Conventional drugs for colitis treatment include aminosalicylate, corticosteroids, antibiotics & immunomodulators. 5- Amino salicylic acid having side effects in 30% of the patients. Systemic corticosteroids producing incidence of complication is 4.3%. Antibiotic therapy is beneficial in 70% of the patients & Immunomodulators having 50 to 70% beneficial effects[2]. This report shows that there is no any appropriate treatment available to treat IBD without side effects. So we are searching for a herbal remedy which will show beneficial effects without side effects in experimentally induced colitis in rats.

Punica granatum is a reputed plant in ayurvedic system of medicine; it has Antioxidant[3] activity and it is also reported as NFkb antagonist[4]. In the light of this the present investigation was undertaken to study the potential of *Punica grnatum* in the treatment of inflammatory bowel disease using DNBS induced colitis in rat.

MATERIALS AND METHODS:

Plant material:
Punica granatum Linn. (Syn:Anar, Family: Punicaceae) fruits were obtained from a botanical garden of Ananad. It was identified and authenticated by Dr. G.C. Jadeja, Professor and Head, Department of Agriculture Botany, B.A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India.

***Corresponding author.**
Vinay Darji,
Department of Pharmacology,
Sharda School of Pharmacy, Pethapur,
Gandhinagar-382610, Gujarat, India.
Tel.: + 91-9825629813
E-mail:vinay_pharma05@yahoo.com

Preparation of *Punica granatum* fruit juice:
Fresh & red fruit of *Punica granatum* was cut into small pieces and then fruit pieces were squeezed out. After that squeezed material was filtered through the muslin cloth to get the fresh and thick juice.

Animals:
Male albino wistar rats weighing 250-300 gm were housed in metabolic cages with free access to standard rat chow (diet) and water ad libitum for one week before the experiment. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Animals were divided into 5 different groups with 6 animals in each group. Study period for all these groups was 18 days. Group 1 served as a normal control & received the std diet throughout the experimental period. Group II served as vehicle control group & received 1.6 ml/kg of 50% ethanol intrarectally on 11th day. Experimental colitis was induced by 2, 4 - dinitro benzene sulfonic acid (DNBS).[5] Group III, IV & V were received the DNBS 120 mg/kg intrarectally on 11th day. Group III served as model control group. Group IV & Group V were received Standard- 5-Aminosalicylic acid 100 mg/kg, p.o. & *Punica granatum* fruit juice 4 ml/kg, p.o. respectively for throughout study period.

Evaluation of physical, histological and biochemical parameters:
During study total water intake, food intake & body weight of each group was measured daily. Animals were sacrificed at the end of study period, colon segment was taken from 10 cm proximal to anus, weighed and scored for inflammatory indices, using the scoring formula of colon mucosa damage index (CMDI) & disease activity index (DAI).[6] Colon samples collected at the end of the study were homogenized & centrifuged to get supernatant which was used to assay Malondialdehyde (MDA)[7], Nitric oxide (NO)[8], Myeloperoxidase (MPO)[9], Superoxide dismutase (SOD).[10]

Histopathology:
Sample of colon from one animal of group I, III.IV & V was collected at the end of study for histopathological evaluation. Photomicrograph of the haematoxylin and eosin stained section of rat colon were taken.

Statistics:
All results were expressed as mean ± S.E.M. p=0.05 was considered statistically significant. Statistical difference between the means of the various groups were analyzed using one-way analysis of variance

RESULTS & DISCUSSION:

Of the several animal models of intestinal inflammation, the well-characterized haptene reagent 2,4 dinitrobenzene sulphonic acid (DNBS)-induced colitis resembles human IBD in terms of its various histological features including infiltration of colonic mucosa by neutrophils and macrophages and increased production of inflammatory mediators including T helper 1 profile of cytokines[11].Therefore in the present study DNBS was used for induction of colitis in the rats to determine the effect of *Punica granatum* on inflammatory bowel disease . DNBS caused mucosal damage, as evident by the increase in CMDI & DAI score as compared to normal control. This increase in CMDI and DAI score was significantly reversed on treatment with *Punica granatum* (Table 1). Induction of inflammatory bowel disease by DNBS was further supported by decrease in water intake, food intake, body wt & increased Colon wt in the model control group animals as compared to the control group animals. *Punica granatum* showed improvement in above physical parameters as compared to the model control group (Table 1). Vehicle control group showed no significant change in histological as well as physical parameters as compared to the control group animals (Table 1).

Table 1. Effect of *Punica granatum* on Histological & Physical parameters

Group	Histological parameters(n=6)			Physical parameters(n=6)		
	CMDI (Grade)	DAI (Grade)	Water intake ml/day/gp	Food intake gm/day/gp	%Body weight decrease	Colon weight mg/rat
Normal control	0.33±0.21	0.17±0.1667	166.38±2.68	134.16±1.907	3.58712±1.044	1739.67±47.64
Vehicle control	1.5±0.22	1.67±0.21	159.72±3.465	130.56±2.057	1.7333±0.4459	1744.17±40.68
Model control	3.5±0.22*	3.83±0.17*	138.06±9.61**	110.5556±7.32	7.23±0.7604*	2341.83±76.07*
Std	1.83±0.31#	2.00±0.26##	156.39±4.81	126.94±3.361##	1.72±0.4458#	1891.67±50.89#
Test	2.33±0.21#	2.50±0.22##	147.22±7.51	122.22±4.146	3.15±0.34#	2023.50±49.8##

Values are expressed as mean ± S.E.M.
** Significantly different from Normal control group at p < 0.001.*
***Significantly different from Normal control group at p < 0.05.*
 # Significantly different from DNBS control group at p < 0.001.
 # #significantly different from DNBS control group at p < 0.05

DNBS model of IBD has been found to be associated with an overproduction of nitric oxide (NO) because of the expression of the inducible isoform of NO synthase (iNOS)[12]. In an inflammatory focus, NO may react with superoxide anion, resulting in oxidative tissue damage through production of peroxynitrite, which is believed to mediate many of the destructive effects of NO in colon inflammation[13]. Thus NO is responsible for oxidative stress which is associated with increased levels of MPO which catalyses the conversion of proportionally more stable hydrogen peroxide to unstable hydrochlorus acid. Additionally MPO induces neutrophil infiltration on mucosal area causing further damage to the tissue[14]. Malondialdehyde is final product of oxidative stress and is good indicator for extent of oxidative stress[15]. Preventive anti-oxidant, such as superoxide dismutase (SOD) enzyme is the first line of defense against reactive oxygen species[16]. Superoxide dismutase (SOD) is widely distributed in cells with high oxidative metabolism and has been proposed to protect such cells against the deleterious effect of superoxide anion[17].

Table 2. Effect of *Punica granatum* on biochemical parameters.

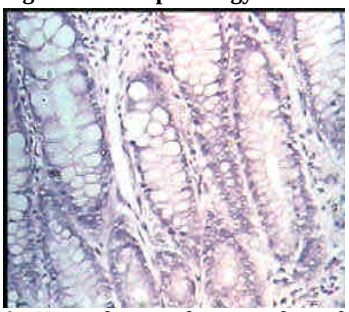
Group	Biochemical parameters(n=6) MDA µg/ml	NO µmoles/ml	MPO nmoles/ml	SOD U/gm of tissue
Normal control	0.16±0.016	353.85±41.11	21.032±1.527	13.46±2.909
Vehicle control	0.18±0.012	370.94±55.61	25.55±3.95	13.33±2.26
Model control	1.35±0.15*	1202.85±71.09*	66.87±3.24*	3.89±1.66**
Std	0.311±0.022#	550.43±80.05#	28.39±3.953#	12.22±1.84##
Test	0.46±0.046#	704.27±53.51#	37.92±4.56#	9.82±1.92##

Values are expressed as mean ± S.E.M.
** Significantly different from Normal control group at p < 0.001.*
***Significantly different from Normal control group at p < 0.05.*
 # Significantly different from DNBS control group at p < 0.001.
 # #significantly different from DNBS control group at p < 0.05

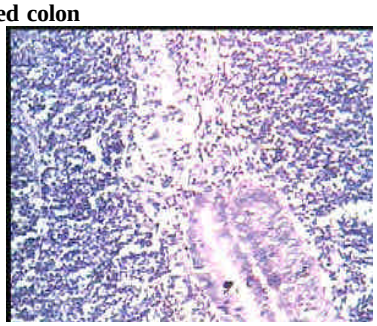
In the present study DNBS model control group showed elevated levels of NO, MPO, MDA and decreased levels of SOD as compared to control group animals, suggesting the possible role of oxidative stress in the induction of colitis (Table 2). Treatment with *Punica granatum* decreased NO level thus suggesting that reduction in iNOS generation may be among the mechanisms responsible for the anti-inflammatory effect of it. Furthermore *Punica granatum* treatment also prevents to increase the levels of MPO & MDA. Anti oxidant defenses were strengthened by treatment with *Punica granatum* as revealed by increase in SOD level as compare to the model control group animals (Table 2).

The most important microscopic findings in human inflammatory bowel disease are the loss of mucus[18], crypt abscess[19] and glandular distortion[20]. Photomicrograph of the haematoxylin and eosin stained section of rat colon showed that DNBS significantly affect the cell structure of the colon. There was rupture of Goblet cells, inflammatory damages to the mucosal layers & inflammatory cellular infiltration observed in the colon of DNBS control animals as compared to normal control group animals. These changes were significantly prevented by standard as well as the test drug *Punica granatum* (Figure 1).

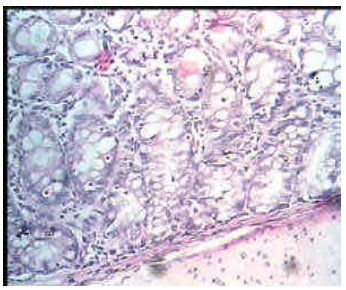
Figure: 1 Histopathology of isolated colon



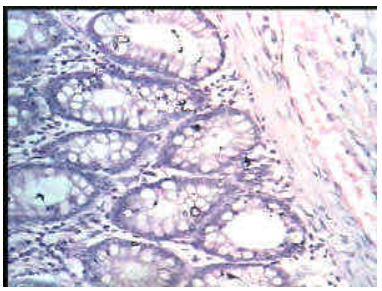
A: Normal control group showed intact epithelial surface;×100.



B: Model control group showed massive necrotic destruction of epithelium,submucosal oedema, haemorrhages and inflammatory cellular infiltration;×100.



C: 5- Amino salicylic acid treated group;×100



D: *Punica granatum* treated group showed protective action against DNBS induced damage;×100

CONCLUSION:
The present study proved the potential effect of *Punica granatum* in inflammatory bowel disease. It may be due to its antioxidant & anti inflammatory activity.

ACKNOWLEDGEMENT:
Dr. G.C. Jadeja, Professor and Head, Department of Agriculture Botany, B.A. College of Agriculture, Anand Agricultural University, Anand for authantification of the *punica granatum* fruits. Sun pharmaceuticals industries ltd. for providing gift sample of 5- Amino salicylic acid.

REFERENCES:

1. Deniel K, Podolsky M.D. Inflammatory bowel disease, *New England journal of medicines*, Aug 8, 2002, vol 347, No. 6.
2. V. alin botoman, M.D, Gregory F. Bonner, M.D. Cleveland Clinic Florida, Ft. Lauderdale, Florida , Daniela A. Botoman, M.D.. North Broward Hospital District Family Practice Program of the University of South Florida, Coral Springs, Florida, *American family physician*, January 1, 1998.Vol. 57, No. 1.
3. Ricci d, Giamperi L, Bucchini A,fraternale D. Antioxidant activity of *Punica granatum* fruits.Fitoterapia 2006 Jun;77(4):310-12.

4. Adams LS, Seeram NP, Aggarwal BB,Takada Y,Sand D, Heber D Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. J agric food chem. 2006 Feb 8; 54(3):980-5.
5. Cuzzocrea S, McDonald M C, Mazzon E. Calpain inhibitor I reduce colon injury caused by dinitrobenzene sulphonic acid in the rat. *Gut* 2001; 48:478-488.
6. Wei-Guo, Shao-Ping L, Bao-Ping Y.Ameliorative Effects of sodium ferulate on experimental induced colitis *World Journal of Gastroenterology* 2003:9 2533-2538.
7. Ohkawa I, Ohisi N, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem* 1979; 95:351-358.
8. Lisa A ,Rindnour, Julia E, Micheal A H et al. A Spectrophotometric method for the direct detection and quantification of nitric oxide, nitrates and nitrite in cell culture media. *Analytical Biochemistry* 2000; 281,223-229.
9. Chunxiang, Zhang, Lisa K et al. Interaction of myeloperoxidase with NADPH oxidase derived reactive oxygen species in vasculature :implication for vascular defence. *Am J Physio Circ physio* 2003; 285 H2563-2572.
10. Misra HP, Fridovich I et al. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; 247:3170 – 3175.
11. Parronchi P., Romagnani P., Annunziato F., Sampugnaro S., Becchio A., Giannarini L., Maggi E., Pupilli C., Tonelli F. & Romagnani S et al. Type 1 helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. *Am. J. Pathol* 1997, 150, 823 – 832.
12. Nathan C et al. Nitric oxide as a secretory product of mammalian cells. *faseb j*, 1996, 3051 – 3064.
13. McCafferty DM. Peroxynitrite and inflammatory bowel disease. *Gut* 2000; 46:436–9.
14. Sekizuka E, Grisham MB, Li MA, Deitch EA, Granger DN et al. Inflammation-induced intestinal hyperemia in the rat: role of neutrophils. *Gastroenterology.* 1988; 95(6):1528–1534.
15. Cheesman, K.H et al. Lipid peroxidation in biological systems. In: Halliwell, B., Aruoma, O.I. (Eds.), DNA and Free Radicals. Ellis Horwood, London, 1993 pp. 12–17.
16. Halliwell, B., Antioxidant characterization: methodology and mechanism. *Biochem. Pharmacol.* 1995, 49, 1341–1348.
17. Fridovich I et al. Superoxide dismutases. *Ann Rev Biochem* 1975; 44:147– 59.
18. Surawicz cm: Mucosal biopsy diagnosis of colitis. Semin Colon Rectal Surg 3: 157-159, 1992.
19. Le berre n, heresback o, kerbaol m, kerbaol m, caulet s, bretagne jf, chaperon j,gosselin m, ramee mp: histological discrimination of idiopathic inflammatory bowel disease from other types of colitis. *j clin pathol* 1995,48: 749-753.
20. Seldenrijk ca, morson bc, meuwissen sgm, schipper nw, lindermand j, meijer cjlm: histopathological evaluation of colonic mucosal biopsy specimens in chronic inflammatory bowel disease; diagnostic implications. *Gut*, 1991, 32: 1514-1520.

Source of support: Nil, Conflict of interest: None Declared