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Evaluation of Potential Effect of *Terminalia Arjuna* against Inflammatory Bowel Disease

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Abstract

Terminalia arjuna wight & Arn. is a deciduous and evergreen tree, belongs to Combretaceae family. In the present study, *Terminalia arjuna was* tested for 2, 4 - dinitro benzene sulfonic acid (DNBS) induced colitis, and its antioxidant & anti inflammatory activities were evaluated to clarify its possible mode of action. Male albino wistar rats were randomly divided into six groups: Normal control (Group I), Model control, colitis induced by DNBS without any therapy (Group II), Vehicle control (Group III), Standard control, treatment with Dexamethasone (Group IV), Treatment with Ethanolic extract of *T.arjuna* (Group V) Treatment with Aqueous extract of *T.arjuna* (Group VI). Treatment was given for 20 days. Rats were sacrificed on the 20th day after the procedure. Malondialdehyde (MDA), Nitric oxide (NO), Reduced Glutathione (GSH), Superoxide dismutase (SOD), activity were measured in the isolated colon tissue. MDA & NO levels in colon tissue homogenate were decreased & GSH, SOD levels were increased in Group IV, Group V & Group VI as compare to those of Group II. There was also increase in food intake, water intake, % body weight & decreased colon weight in Group IV, Group V & Group VI as compared to Group II. There was also improvement in inflammatory indices of colon mucosal damage index (CMDI) & Disease activity index (DAI) & histopathology of Group IV, Group V & Group VI as compared to those of group II. The results of our study suggest that *T.arjuna* therapy has beneficial effects on the course of experimental colitis.

Keywords: *Terminalia arjuna*, Inflammatory bowel disease (IBD), Antioxidant, Anti inflammatory

Contents

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1. 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 immuno inflammatory 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 and 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. *Terminalia arjuna* is a reputed plant in ayurvedic system of medicine; it has Antioxidant [3], Anti inflammatory [4], Antimicrobial [5] Anti fungal [6], Anthelmintic [7], Anti cancer [8], Anti diabetic [9], Cardiotonic [10], Gastroprotective [11] and Hypolipidemic [12] activities reported. On the basis of this, the present investigation was undertaken to study the potential of *Terminalia arjuna* in the treatment of inflammatory bowel disease using DNBS induced colitis in rat.

2. Materials and Methods

Plant material: *Terminalia arjuna* (Family: Combretaceae) bark was obtained from LVG, ahmedabad. It was identified and authenticated by macroscopy, microscopy, various chemical tests and analysis.

Preparation of Aqueous and ethanolic extracts:

The shady dried plant materials were subjected to pulverization to get coarse powder which was defatted using petroleum ether and sequentially extracted with 90% ethanol and double distilled water in soxhlet apparatus. The extracts were filtered and concentrated to dryness in rotary evaporator (microwave oven). The obtained crude extracts were stored at 2-8℃ until used in the assay.

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 06 different groups with 06 animals in each group. Study period for all these groups was 20 days. Group I served as a normal control & received the standard diet throughout the experimental period. Experimental colitis was induced by 2, 4 – di nitro benzene sulfonic acid (DNBS) [13]. Group II, IV, V & VI were received the DNBS 120 mg/kg intrarectally. Group II served as model control group. Group III served as vehicle control group & received 1.6 ml/kg of 50% ethanol intrarectally. Group IV was received Standard Dexamethasone 02 mg/kg, i.p. While Group V & Group VI were received *Terminalia arjuna* Ethanolic extract 200 mg/kg p.o. and Aqueous extract 200 mg/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) [14]. Colon samples collected at the end of the study were homogenized & centrifuged to get supernatant which was used to assay Malondialdehyde (MDA) [15], Nitric oxide (NO) [16], Reduced Glutathione (GSH) [17], Superoxide dismutase (SOD) [18].

Histopathology: Sample of colon from one animal of all the groups 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. $p \leq 0.05$ was considered statistically significant. Statistical difference between the means of the various groups was analyzed using one-way analysis of variance.

3. Results and 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 [19].Therefore in the present study DNBS was used for induction of colitis in the rats to determine the effect of *Terminalia arjuna* 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 ethanolic as well as aqueous extracts of *Terminalia arjuna* (Figure-1&2).

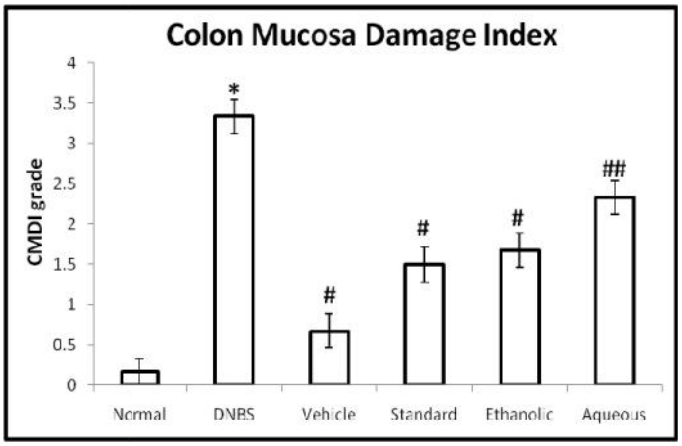


Figure 1: Effect of Terminalia arjuna extracts on CMDI in DNBS treated rats

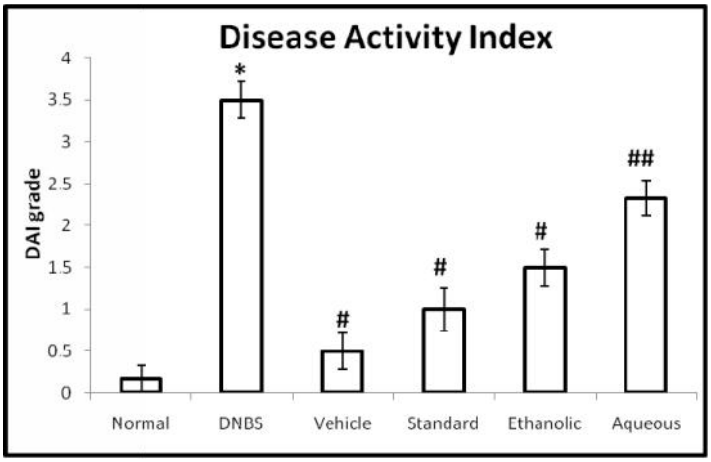


Figure 2. Effect of Terminalia arjuna extracts on DAI in DNBS treated rats

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 normal control group animals. Both the extracts of *Terminalia arjuna* showed improvement in above physical parameters as compared to the model control group (Figure-3, 4, 5 &6). Vehicle control group showed no significant change in histological as well as physical parameters as compared to the control group animals (Figure- 1 to 6).

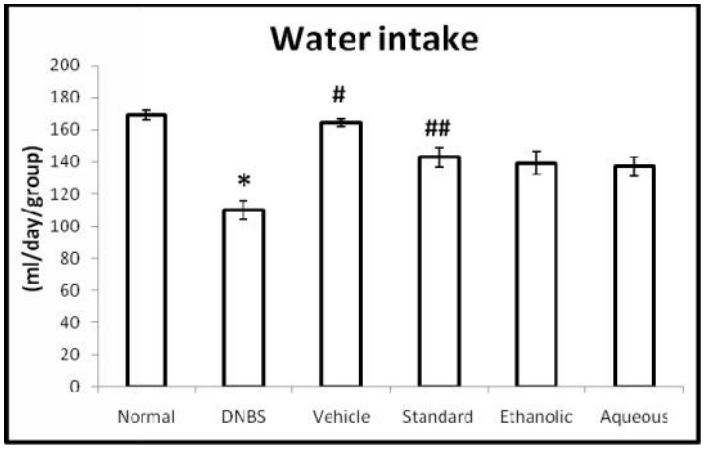


Figure 3: Effect of Terminalia arjuna extracts on water intake in DNBS treated rats

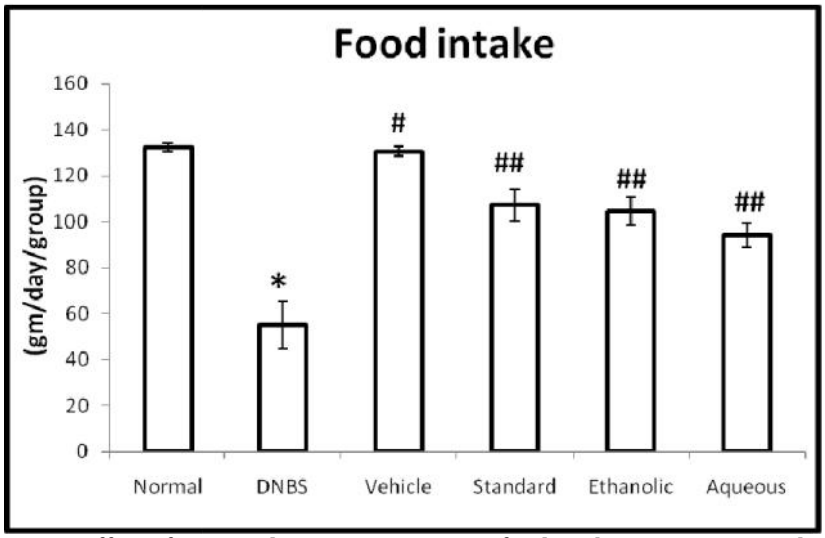


Figure 4: Effect of Terminalia arjuna extracts on food intake in DNBS treated rats

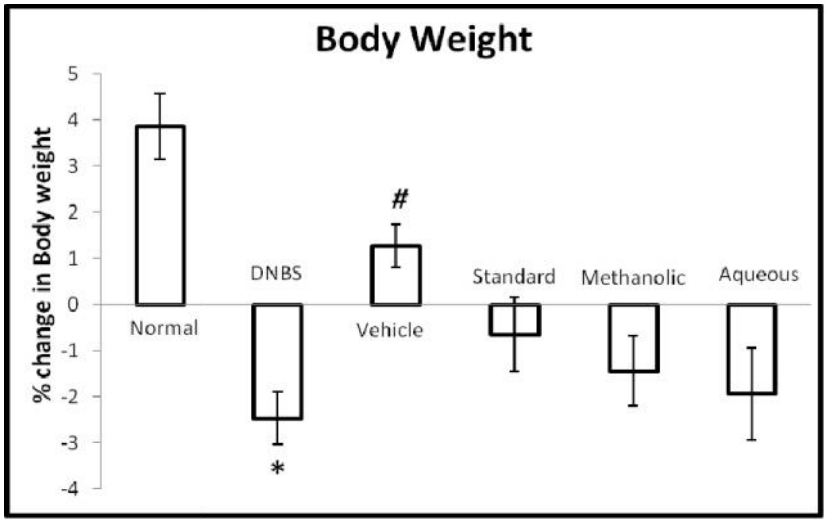


Figure 5: Effect of Terminalia arjuna extracts on body weight in DNBS treated rats

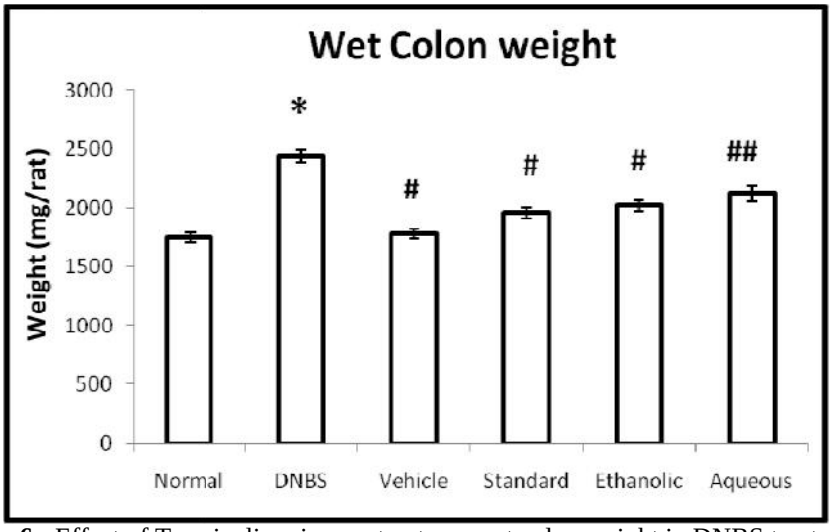


Figure 6: Effect of Terminalia arjuna extracts on wet colon weight in DNBS treated rats

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) [20]. 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 [21]. Thus NO is responsible for oxidative stress. Malondialdehyde is final product of oxidative stress and is good indicator for extent of oxidative stress [22]. Preventive anti-oxidant, such as superoxide dismutase (SOD) enzyme is the first line of defense against reactive oxygen species [23]. 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 [24]. Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation. During oxidative stress, GSH gets oxidized and cannot be regenerated [23].

In the present study DNBS model control group showed elevated levels of NO & MDA and decreased levels of SOD & GSH as compared to normal control group animals, suggesting the possible role of oxidative stress in the induction of colitis (Figure-7 to 10). Treatment with ethanolic and aqueous extracts of *Terminalia arjuna* decreased NO level thus suggesting that reduction in iNOS generation may be among the mechanisms responsible for the anti-inflammatory effect of it. Furthermore both the extracts of *Terminalia arjuna* also prevent to increase the MDA level. Anti oxidant defenses were strengthened by treatment with ethanolic as well as aqueous extracts of *Terminalia arjuna* as revealed by increase in SOD & GSH levels as compare to the model control group animals (Figure-7 to 10).

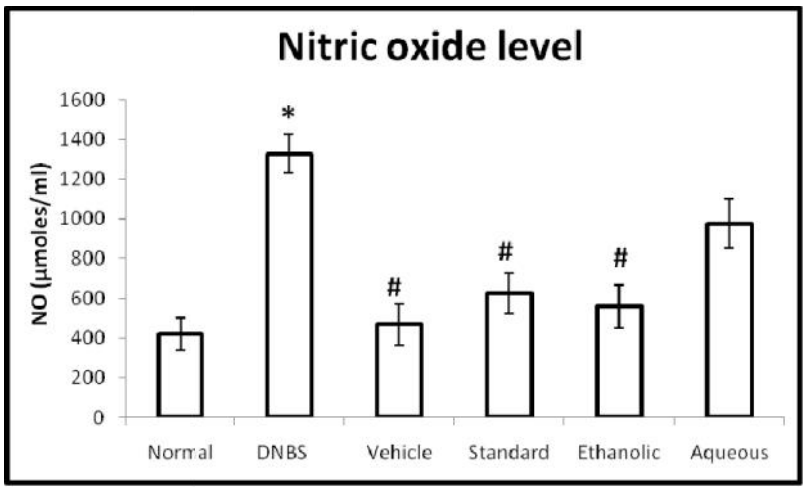


Figure 7: Effect of Terminalia arjuna extracts on NO in DNBS treated rats

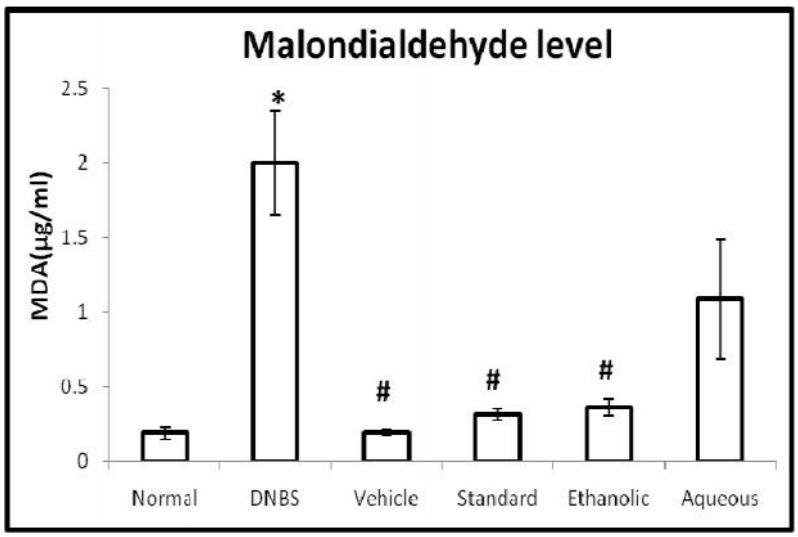


Figure 8: Effect of Terminalia arjuna extracts on MDA in DNBS treated rats

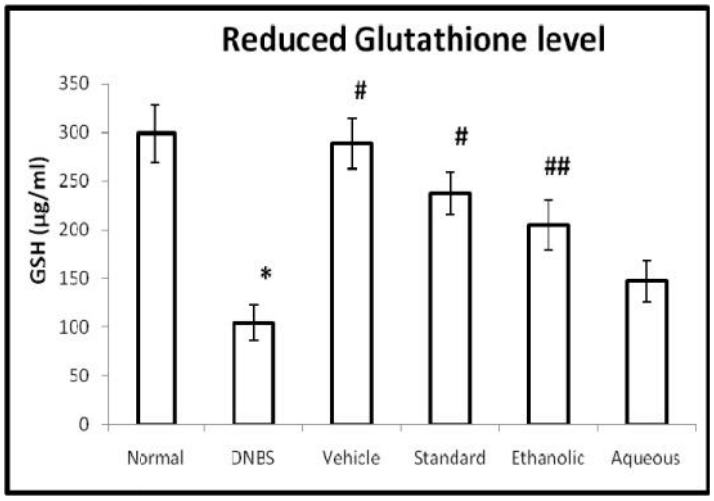


Figure 9: Effect of Terminalia arjuna extracts on GSH in DNBS treated rats

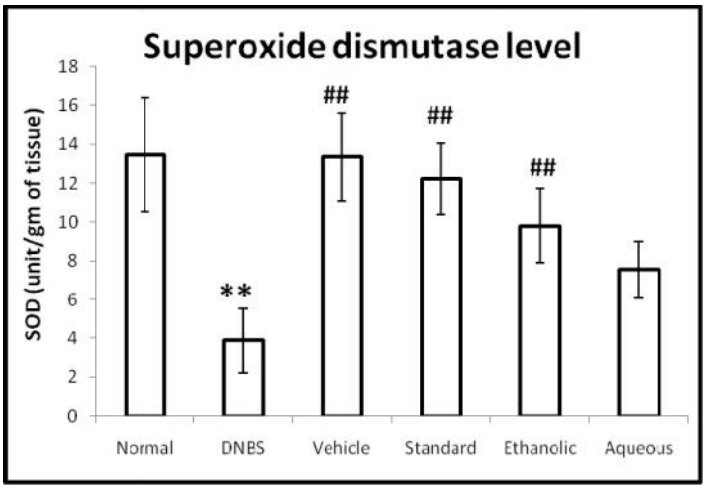
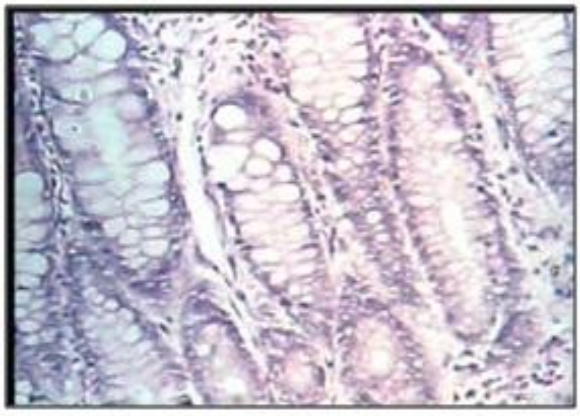
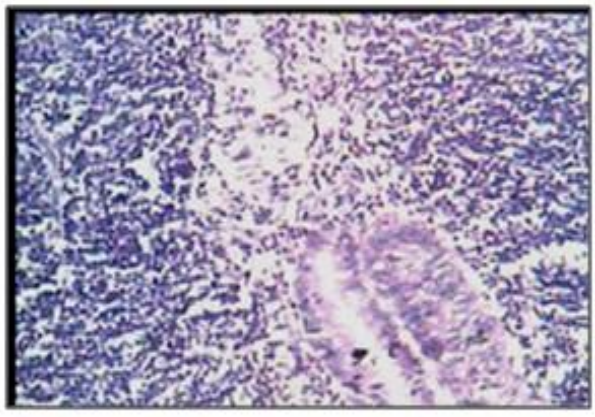


Figure 10: Effect of Terminalia arjuna extracts on SOD in DNBS treated rats

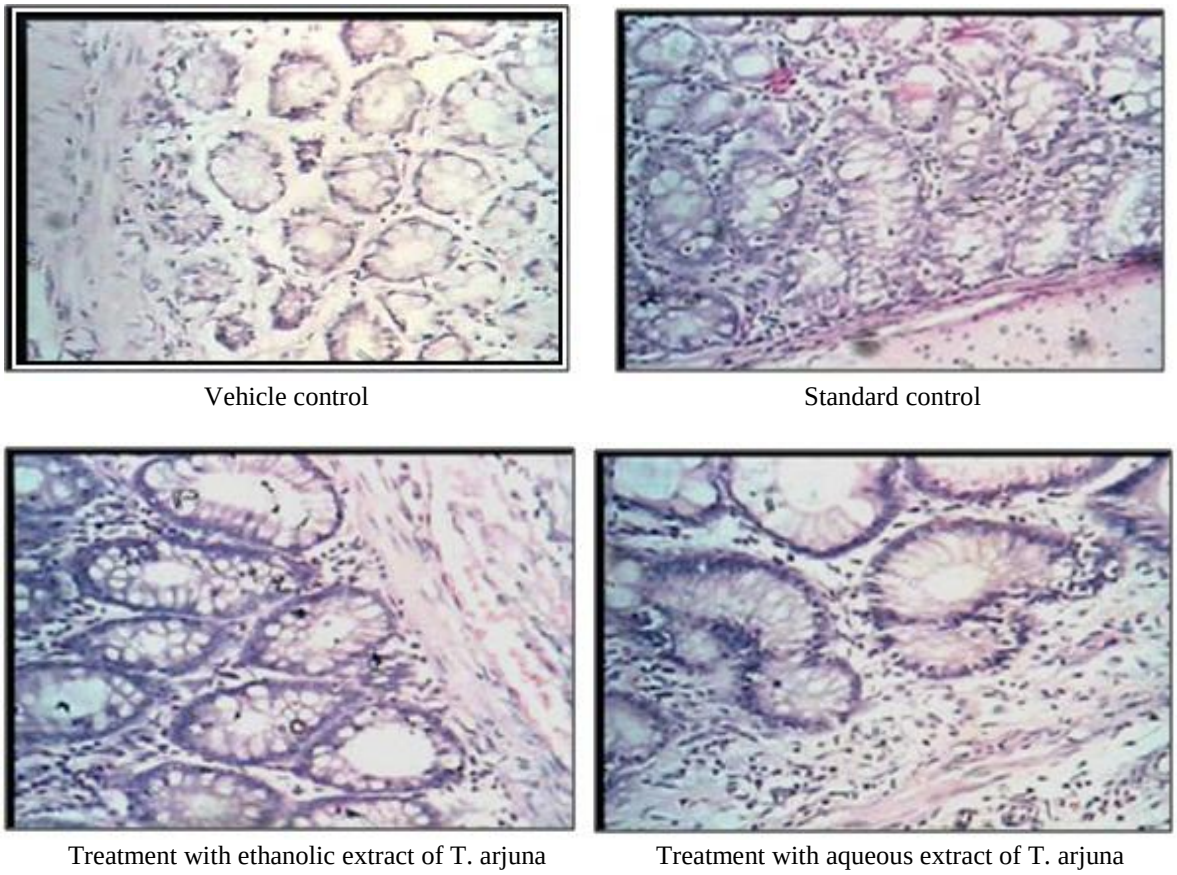
The most important microscopic findings in human inflammatory bowel disease are the loss of mucus [25], crypt abscess [26] and glandular distortion [27]. Photomicrograph of the haematoxylin and eosin stained section of rat colon showed that DNBS significantly affect the cell structure of the colon. There 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 both the extracts of the test drug *Terminalia arjuna* (Figure 11).



Normal control



DNBS control (120 mg/kg)



Vehicle control | Standard control

Treatment with ethanolic extract of T. arjuna | Treatment with aqueous extract of T. arjuna

Figure 11: Histopathology of rat colon

4. Conclusion

The present study proved the potential effect of *Terminalia arjuna* in inflammatory bowel disease. It may be due to its antioxidant & anti inflammatory activity. In addition, ethanolic extract is found to be better than aqueous extract. However, it requires further investigation to establish the exact mechanism of both the extracts of *Terminalia arjuna* in antagonizing DNBS activity in colon.

5. References

1. K. Deniel, MD Podolsky. Inflammatory bowel disease, *New Eng J of Med*. **2002**, 347: 6.
2. VA Botoman, GF Bonner, DA Botoman, North Broward Hospital District Family Practice Program of the University of South Florida, Coral Springs, Florida, *American family physician.* **1998**, 57: 1.
3. P. Doorika. and T. Ananthi. Antioxidant and Hepatoprotective properties of Terminalia arjuna Bark on Isoniazid Induced Toxicity in Albino rats. *Asian J. Pharm. Tech.* **2012**, 2(1): 15-8.
4. S. Halder, N. Bharat, PK Mediratta, I. Kaur & KK Sharma. Anti-inflammatory, Immunomodulatory and anti nociceptive activity of Terminalia arjuna Roxb bark powder in mice and rats. *Ind J of Exp Biol***. 2009**, 47: 577-83.
5. KR Aneja, C Sharma, R Joshi. Antimicrobial activity of Terminalia arjuna Wight & Arn.: an ethnomedicinal plant against pathogens causing ear infection. *Braz J Otorhinolaryngol*. **2012**, 78(1): 68-74.
6. R. Nema, P. Jai, S Khare, A. Pradhan, A. Gupta, D. Singh. Antibacterial and antifungal activity of Terminalia Arjuna leaves extract with special reference to Flavanoids. *Basic Res J of Med and Clinl Sciences.* **2012**, 1(5): 63-5.
7. HA Bachaya, Z Iqbal, MN Khan, A Jabbar, AH Gilani and IU Din. In vitro and In vivo anthelmintic activity of Terminalia arjuna bark. *Int. J. Agric. Biol.*, **2009**, 11: 273–8.
8. M Biswas, AK Ghosh, PK Haldar. Anti-leishmanial and Anti-cancer Activities of a Pentacyclic Triterpenoid Isolated from the Leaves of Terminalia arjuna Combretaceae. *Trop J of Pharm Res.* **2010**, 9 (2): 135-40.
9. S. Barman and S. Das. Hypoglycemic effect of ethanolic extract of bark of Terminalia arjuna Linn. in normal and alloxan‐induced noninsulin-dependent diabetes mellitus albino rats. *Int J of Green Pharmacy*. **2012**, 279-84.

10. K. Karthikeyan, BR Sarala Bai, K Gauthaman, KS Sathish, SN Devaraj. Cardioprotective effect of the alcoholic extract of Terminalia arjuna bark in an in vivo model of myocardial ischemic reperfusion injury. *Life Sciences*. **2003**, 73: 2727–39.
11. RS Devi, S Narayan, S Vani, CS Devi. Gastroprotective effect of Terminalia arjuna bark on diclofenac sodium induced gastric ulcer. *Chem Biol Interact.* **2007**, 167(1): 71-83.
12. S Sharma, D Sharma and N. Agarwal. Diminishing effect of arjuna tree (Terminalia arjuna) bark on the lipid and oxidative stress status of high fat high cholesterol fed rats and development of certain dietary recipes containing the tree bark for human consumption. *Research in Pharmacy*. **2012**, 2(4): 22-30.
13. **S Cuzzocrea, MC McDonald, E Mazzon.** Calpain inhibitor I reduce colon injury caused by dinitrobenzene sulphonic acid in the rat. *Gut*, **2001**, 48: 478-88.
14. Wei-Guo, L Shao-Ping, Y. Bao-Ping. Ameliorative Effects of sodium ferulate on experimental induced colitis *World Journal of Gastroenterology*, **2003,** 9: 2533-8.
15. I. Ohkawa, N. Ohisi, N.Yagi. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem*, **1979**, 95: 351-8.
16. A. Lisa, Rindnour, E.Julia, AH Micheal 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: 223-29.
17. MS Moron, JW Depierre and B Mannervik. Levels of glutathione, glutathione reductase and glutathione S transferase activities in rat lung and liver. *Biochem Biophys Acta,* **1979**, 582: 67-78.
18. HP Misra, I Fridovich et al. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*, **1972**, 247: 3170-5.
19. P. Parronchi., P. Romagnani., F. Annunziato, S. Sampugnaro, A.Becchio, L. Giannarini, 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: 823-32.
20. C Nathan et al. Nitric oxide as a secretory product of mammalian cells. *Faseb J*, **1996**, 3051-64.
21. DM McCafferty. Peroxynitrite and inflammatory bowel disease. *Gut*, **2000**, 46: 436–9.
22. KH Cheesman et al. Lipid peroxidation in biological systems. In: Halliwell, B., Aruoma, O.I. (Eds.), DNA and Free Radicals. Ellis Horwood, London, **199**3, 12–17.
23. B. Halliwell,, Antioxidant characterization: methodology and mechanism. *Biochem. Pharmacol.***1995**, 49: 1341-8.
24. I. Fridovich et al. Superoxide dismutases. *Ann Rev Biochem*, **1975**, 44: 147-59.
25. CM Surawicz. Mucosal biopsy diagnosis of colitis. *Semin Colon Rectal Surg*. **1992**, 3: 157-9.
26. N Le berre, O Heresback, M Kerbaol, S Caulet, JF Bretagne, J Chaperon et al. Histological discrimination of idiopathic inflammatory bowel disease from other types of colitis. *J Clin Pathol*, **1995**, 48: 749-53.
27. CA Seldenrijk, BC Morson, SGM Meuwissen, NW Schipper, J Lindermand, CJ Meijer. Histopathological evaluation of colonic mucosal biopsy specimens in chronic inflammatory bowel disease; diagnostic implications. *Gut*, **1991**, 32: 1514-20.