INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-4, Issue-2, April-June-2013 Coden : IJABPT Copyrights@2013

ISSN : 0976-4550

Received: 30th Jan-2013 *Revised: 15th Feb-2013* *Accepted: 16th Feb-2013*

Research article

COMPARATIVE EFFICACY OF DIFFERENT REFERENCE DRUGS ON TRINITROBENZENESULFONIC ACID-INDUCED ULCERATIVE COLITIS IN THE RAT MODEL

P.S.Venkatesan[1], M. Deecaraman[1] and M. Vijayalakshmi[1]

[1]Department of IBT, Dr. M.G.R. Educational & Research Institute and University, Maduravoyal, Chennai – 600095

Corresponding Author: P.S.Venkatesan, Email:venkyvet74@gmail.com

ABSTRACT: Crohn's disease and Ulcerative colitis were chronic inflammatory disorders of the bowel categorized as inflammatory bowel diseases. Trinitrobenzene sulfonic acid (TNBS)-induced colitis was one of the most common methods for studying inflammatory bowel disease in animal models. Several factors may, however, affect its reproducibility, rate of animal mortality, and macroscopic and histopathological outcomes.The current study was undertaken with the objective to validate the main contributing factors to this method and compare the effects of different reference drugs upon better amelioration of trinitrobenzenesulfonic acid (TNBS) induced colitis. With the above objectives, ulcerative colitis was induced by intrarectal administration of TNBS in male Wistar rats at a dose rate of 20 mg in 0.5 mL of ethanol per animal for all groups except the negative control group, which received 0.5 mL of normal saline. Different reference drugs like dexamethasone (1 mg/kg, intraperitoneally (i.p.) and 2 mg/kg, orally (p.o.)), hydrocortisone acetate (20 mg/kg, i.p.; 20 mg/kg, enema) and sulfasalazine 500mg/kg ,p.o.were administered daily once from Day 3 to 9 except the negative and positive controls which received normal saline at the rate of 10 mL/kg body weight. All the animals were sacrificed on Day 10; the colons were excised and the colon morphology and net weight of the colon segment were graded and measured, respectively. The intestinal damage had improved significantly in the experiment groups that received different reference drugs which is comparable with sulfasalazine treated group. The experimental observations, gross pathology of intestinal lesions and statistical analysis reveals no significant difference among the different reference drugs treated groups.

Keywords: Reference drugs, trinitrobenzenesulfonic acid, Hydrocortisone, Dexamethasone, Sulfasalazine

INTRODUCTION:

Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD) which affects intestinal tract, specifically the large intestine or colon that includes characteristic ulcers, or open sores, in the colon. The main symptom of the active disease is usually constant diarrhea mixed with blood, with a gradual onset. Although the symptoms of ulcerative colitis can sometimes diminish by themselves, it requires treatment to prevent re occurence. It is similar to Crohn's disease, another form of IBD. The causative reasons for ulcerative colitis has unknown, presumed genetic component, dietary modification and the disease may be triggered in a susceptible person by environmental factors (Stephen 2001). Although dietary modification may reduce the discomfort, it is not due to dietary factors. No medical cure has been developed for IBD and treatment focuses on producing and maintaining remission (Teo and Tam 2005, Leifert et al 1995 and Ahimou et al 2000). Conventional pharmacotherapy for both types of IBD is treatment with aminosalicylates and corticosteroids (Bais et al 2004 and Liu et al 2007). Moreover, Immunosuppressive agents and biological response modifiers are considered as alternative therapies (cavaglieri et al 2005). Nonetheless, available medicines are not universally effective and result in marked deleterious effects (Mohammadipour et al 2009).

These challenges have thus heightened the need for research in order to adopt new therapeutic approaches for the treatment of IBD. Some times colectomy is necessary, and is considered to be a cure for the disease (Sands 2000). However, the experimental animal models couldn't fully reflect the complexity of the disease present in human (Ngugia et al 2005) and each method has its own disadvantages. Morris and coworkers constructed a simple and reproducible rat model of acute and chronic colonic inflammation and ulceration by means of intracolonical injection of 2,4,6- trinitrobenzenesulfonic acid (TNBS) in ethanol (Peys et al 2007). The major advantages of this model include proposing a simple process and reproducible colonic damage, short duration of the experiment, long-lasting damage accompanied by inflammatory cell infiltration and ulcers (Selvam et al 2009). In addition, this model mimics both acute and chronic phases of ulcerative colitis which is one of the hallmarks of human ulcerative colitis. There is compelling evidence that dysregulation of the mucosal immune system is a major contributing factor to the pathogenesis of IBD. In this regard, a few murine models of IBD have shown that alterations in immune system functions result from a failure of regulation by T-helper cells and lead to acute and chronic inflammation in the intestine. In comparison with other animal models of IBD, TNBS is an efficient method. It can mimic the pattern of inflammation with human IBD and is widely applicable to mice, rats and guinea pigs (Sharma et al 2008 and Fiorini et al 1985). On the other hand, TNBS model of colitis suffers from some disadvantages (e.g.the absence of spontaneous relapse which is the hallmark of human ulcerative colitis) as do many other methods. In addition, the reproducibility of the model is dependent upon the dose of TNBS (Muscettola et al 1992). Corticostroids and sulfasalazine are selected as reference drugs in most studies conducted on colitis. However, their efficacy in remission of experimental ulcerative colitis is sometimes controversial and has not been compared based on various routes of administration (i.e. i.p., p.o. or enema) (Teo et al 2003 and Henriques et al 1998). Therefore, the present study was set out to determine the optimum dose of TNBS under our laboratory conditions and therapeutic effects of some reference drugs in remission of this immune-based animal model of IBD.

MATERIALS AND METHODS

Chemicals and Reagents: 2,4,6-Trinitrobenzenesulfonic acid was purchased from Sigma-Aldrich. Other reagents were purchased in India.

Animals: Ten to eleven week old male Wistar rats (200 – 250 gm) were obtained from the Gentox Bioscience (India Private Limited, Hyderabad). They were housed in polypropylene cages and maintained in an air-conditioned animal facility with a 12 h light–dark cycle. Animals were provided with free access to reverse osmosis-filtered water and feed procured from provimi, India. This study was carried out with the IAEC approval and in accordance to CPCSEA guidelines.

Experimental design: Animals were randomly assigned to one of seven groups (Table 1). To investigate a possible influence of different reference drugs

Table 1. Study Design

Group No.	Group	Drug	No. of animals
1	Negative control	Saline intrarectally Day 1 + Control Vehicle (saline) at 10 mL/kg orally/ TID from Day 3 - 9	6
2	Positive control for UC	TNBS Intrarectally Day 1 + Control Vehicle (saline) at 10 mL/kg orally/ TID from Day 3 - 9	6
3	Dexamethasone (1mg/kg, i.p.)	TNBS intrarectally Day 1 + Dexamethasone 1mg/kg i.p. once daily from Day 3 - 9	6
4	Dexamethasone (2 mg/kg, p.o.)	TNBS intrarectally Day 1 + Dexamethasone 2mg/kg orally once daily from Day 3 - 9	6
5	Hydrocortisone (20mg/kg, i.p.)	TNBS Intrarectally Day 1 + Hydrocortosone 20mg/kg i.p. once daily from Day 3 - 9	6
6	Hydrocortisone (20mg/kg, enema)	TNBS Intrarectally Day 1 Hydrocortisone 20mg/kg,enema once daily from Day 3 - 9	6
7	Sulfasalazine	TNBS Intrarectally Day 1 + Sulfasalazine 500 mg/kg orally once daily from Day 3 – 9	6

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Induction of colitis: Briefly, animals were fasted overnight and 2,4,6- trinitrobenzenesulfonic acid was diluted with 50 % ethanol (v/v) to attain a concentration of 40 mg/mL. TNBS was administered on Day 1 by intrarectal route using a 2 mL syringe attached to a 10 cm polyethylene catheter at a dose of 20 mg per animal. After administration, the rats were held upside down for approximately 30 seconds to prevent immediate leakage of the agent from the anus.
Test item preparation and administration: All reference drugs were prepared freshly on each day before the start of the dosing and administered in a day at the rate of dose mentioned in Table 1 from Day 3 up to and including Day 9. Sulfasalazine was administered once daily from Day 3 up to and including Day 9 at the dose rate as mentioned in Table 1. Suspension of reference drugs were freshly prepared using 0.3% Tween 80 in normal saline as a vehicle for p.o. and i.p. administrations.
Observations: Rats were examined for clinical signs once in a day and mortality twice daily during the acclimatization and dosing phases. Body weight of the animals was recorded on the day of TNBS administration (Day 1) and during the treatment period on Days 3, 5, 7 and 9. The amount of feed consumed by the animal was recorded daily. Daily observation of diarrhea was recorded and graded as 1 - Mild, 2 - Moderate, 3 - Severe and 4 – Occult blood.
Measurement of colon weight and assessment of colonic damage: All surviving rats were euthanized on Day 10. A 5 cm segment (from 10 to 5 cm proximal to the anus) of distal colon was removed and placed on an ice-cold plate,cleared of fat and mesentery and blotted on filter paper. The colon samples were weighed as a reflection of colonic edema. Each specimen of large intestine was longitudinally opened and scored for macroscopically visible damage on a scale ranging from 0 – 5 (Table 2) indicative of areas of mucosal discoloration, erosion, exudation, ulceration and bowel wall thickening by an observer unaware of the treatment, according to the criterion previously proposed by (Peys et al, 2007 and Selvam et al 2009)

Table 2. TNBS Colitis Macroscopic Grading Standards.

Grade	Observation
0	No damage
1	No ulceration, localized hyperaemia
2	Ulceration with no significant inflammation
3	Ulceration with inflammation at one site
4	Two or more sites of inflammation and/or ulceration
5	Two or more major sites of ulceration and/or inflammation or one major site of inflammation and ulceration extending 1.5 cm along the colon

Statistical Analysis: The results are expressed as mean±SEM. Differences among means were tested for statistical significance by one-way analysis of variance and a Tukey's multiple comparison test. All statistical analyses were carried out with the Graphpad software. Statistical significance was set at $p<0.05$.

RESULTS

Intracolonic administration of TNBS/ethanol resulted in an inflammatory response characterized by extensive mucosal disruption, linear and deep ulcers, hemorrhage and submucosal edema. Intra-abdominal pathological adhesions between colon and small bowel and other organs were seen in less than 10 % of the rats. Diarrhea and lack of weight gain were evident in all TNBS-induced rats in group 2.
Effect of drugs on body weight: The body weights were recorded on days Days 1, 3, 5, 7 and 9 during the experimental period(Table 3 and Figure 1). Animals were segregated in to seven groups on day Day 1 in such a way that there is no statistical significance in their mean body weight. On Day 3, the animals of groups 2-7 were re-randomized and allocated to six groups (group 2 – 7) according to their per cent body weight gain irrespective of the slight dissimilarity in the mean body weights that had no statistical significance ($P<0.05$). Significant difference ($P<0.05$) was observed between the TNBS treated groups (2, 3, 4, 6, 7) and the saline treated negative control Group 1 up to Day 5.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

This clearly indicates that, the degree of damage in the intestinal tissue and reduced body weight is directly proportional to the intra rectal TNBS infusion. Interestingly on Day 7, there was lots of dissimilarity in mean body weight among different groups (Table 3). There were no statistically significant difference among groups 1, 3 and 5, similarly among groups 2, 3, 4, 6 and 7. But groups 2, 4, 6 and 7 showed statistically significant (P<0.05) differences from Group 1. Similarly, Group 2 and 7 were significantly (P<0.05) different from groups 1 and 5. On Day 9, groups 2, 3, 6 and 7 were significantly (P<0.05) different from Group 1. But there were no statistical difference among groups 1, 4 and 5 and among groups 2, 3, 4, 6 and 7. It is evident that all the drugs are effective in controlling the degree of intestinal inflammation.

Table 3. Effect of drugs on body weight

Group	Mean ± SEM (n = 6)				
	Day 1	Day 3	Day 5	Day 7	Day 9
1	257.28^{a}±4.24	281.35^{a}±6.41	284.98^{a}±5.61	303.90^{a}±5.70	317.70^{a}±6.72
2	257.35^{a}±4.43	250.22^{b}±7.25	258.77^{b}±6.52	266.22^{c}±5.79	276.18^{c}±5.21
3	257.20^{a}±4.39	254.20^{b}±6.13	264.18^{b}±6.63	282.93^{abc}±7.66	291.17^{bc}±8.86
4	257.12^{a}±4.04	252.20^{b}±5.30	262.42^{b}±4.55	280.85^{bc}±5.90	292.12^{abc}±4.92
5	256.87^{a}±4.22	262.97^{ab}±4.81	270.77^{ab}±4.01	295.35^{ab}±3.80	302.31^{ab}±6.92
6	255.97^{a}±3.11	246.68^{b}±4.62	256.82^{b}±4.71	279.03^{bc}±5.39	291.18^{bc}±7.19
7	256.88^{a}±4.23	240.12^{b}±7.79	251.02^{b}±2.82	266.99^{c}±2.50	277.90^{bc}±4.47

Means bearing different superscripts in the same column differ significantly (*P*<0.05). The method currently being used to discriminate among the means is Tukey's HSD procedure.

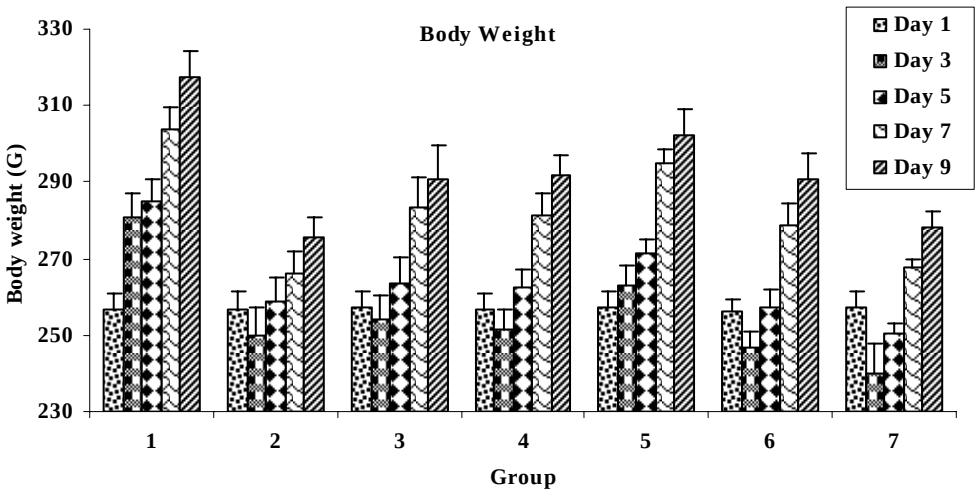

Figure 1. Mean body weight of each group on different days

Effect of drugs on feed consumption: We observed in the present study that TNBS produces substantial effect on feed consumption(Table 4). These results obviously indicate that there is significant (P<0.05) difference on Days 1 and 2 between the group that was rectally administered saline (group 1) and the other groups that received TNBS. From day three onwards, the mean feed consumption slightly varied among the groups. On day 3, the groups 2, 6 and 7 differed significantly (P<0.05) from group 1. Whereas, the group 3, 4 and 5 does not differ statistically (P<0.05) from all other groups. On day 4, group 1 was statistically significantly (P<0.05) different from all other groups except group 3 which does not differ among all other groups. In day 5 and 6, group 2 was significantly (P<0.05) different from group 1. Whereas, remaining all other groups does not differ statistically (P<0.05) compared either with group 1 or 2. On day 7, group 7 that received sulfasalazine was significantly (P<0.05) different from group 1.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Whereas, remaining all other groups does not statistically (P<0.05) differ compared either with group 1 or 7. There were no statistically significant (P<0.05) difference among all the groups on day 8. On day 9, group 3 and 6 does not differ statistically (P<0.05) compared either with other groups, whereas group 2, 4, 5 and 7 were statistically significantly (P<0.05) different from group 1. TNBS treated group 2 showed reduced feed consumption through out the study period. The reduced feed consumption might be due to intestinal inflammation. The mean feed consumption per day in the drug treated rats had significantly improved compared to the TNBS treated groups, which shows the desirable effect of drugs. However, no significant differences were observed between the different drugs through out the study period. Drug treated animals showed significant improvement in the per day mean feed consumption and numerically higher value than the TNBS control groups which indicate that the drugs produce desirable effect.

Table 4. Effect of Drugs on Feed consumption (feed weight in gram)

Group	Feed consumption/Day/Animal (Mean ± SEM; n = 6)								
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
1	27.82^{a}±0.97	21.93^{a}±0.71	28.03^{a}±0.53	24.95^{a}±0.91	22.02^{a}±0.56	23.02^{a}±1.14	24.08^{a}±1.57	23.93^{a}±1.84	22.93^{a}±1.33
2	8.93^{b}±3.06	8.02^{b}±2.08	18.00^{b}±3.37	18.00^{b}±0.90	15.02^{b}±1.17	16.04^{b}±1.22	18.00^{ab}±0.65	17.93^{a}±0.74	16.97^{b}±1.23
3	8.15^{b}±2.22	7.02^{b}±1.80	21.05^{ab}±1.87	21.03^{ab}±1.36	20.12^{ab}±0.83	19.02^{ab}±0.92	20.02^{ab}±1.89	23.10^{a}±2.35	21.03^{ab}±1.52
4	12.98^{b}±2.41	8.95^{b}±1.08	19.90^{ab}±2.01	19.18^{b}±1.72	18.07^{ab}±0.75	18.08^{ab}±0.73	19.95^{ab}±1.64	19.97^{a}±1.89	18.03^{b}±0.91
5	12.02^{b}±1.38	09.93^{b}±1.57	17.95^{ab}±2.95	19.00^{b}±1.58	16.93^{ab}±2.59	16.97^{ab}±2.77	18.02^{ab}±1.33	17.90^{a}±2.33	16.02^{b}±1.18
6	15.05^{b}±3.96	13.91^{b}±2.78	17.90^{b}±1.58	17.92^{b}±0.87	16.97^{ab}±0.94	19.03^{ab}±0.93	20.02^{ab}±1.55	21.90^{a}±2.22	19.92^{ab}±1.15
7	12.07^{b}±2.98	9.35^{b}±2.49	17.00^{b}±3.18	18.00^{b}±1.52	20.08^{ab}±1.93	20.30^{ab}±2.31	17.10^{b}±1.24	17.02^{a}±1.92	16.02^{b}±1.52

Means bearing different superscripts in the same column differ significantly (*P*<0.05). The method currently being used to discriminate among the means is Tukey's HSD procedure.

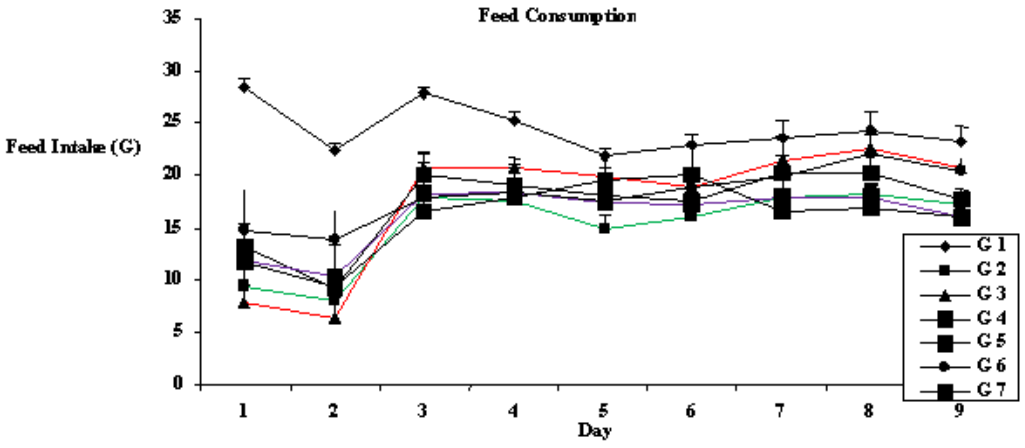

Figure 2. Feed intake of different groups on different days.

Effect of drugs on fecal score: Evidence of diarrhea was noticed in all the groups that received TNBS from Day 2 up to day three (Table 5). On day 4, sulfasalazine treated group 7 showed significant differences (P<0.05) compared with group 2 whereas groups 3 – 6 does not differ statistically (P<0.05) compared to all other groups. From day 5 onwards, there is no statistically significant (P<0.05) difference among all the groups. Signs of diarrhoea were absent in the different formulations of drugs treated groups from day 7 onwards which is similar to the positive drug control sulfasalazine treated group 7 . The diarrhea persisted up to day 8 in the TNBS alone treated positive control group 2. Even though there were no statistical differences among the groups including group 2 from day 5 onwards, numerically there was a comprehensible demarcation in the mean faecal score between group 2 and other groups (3, 4, 5 and 6) but not among the groups 3, 4, 5 and 6.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Table 5. Effect of Drugs on fecal score.

Group	Mean ± SEM (n = 6)								
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	3.00±0.00	3.00±0.00	2.68^{a}±0.24	1.68^{a}±0.22	1.34^{a}±0.22	0.68^{a}±0.24	0.17±0.17	0.00
3	0.00	3.00±0.00	3.00±0.00	2.01^{ab}±0.27	1.51^{a}±0.23	0.51^{a}±0.23	0.34^{a}±0.24	0.00	0.00
4	0.00	3.00±0.00	3.00±0.00	2.32^{ab}±0.22	1.32^{a}±0.24	0.68^{a}±0.22	0.34^{a}±0.22	0.00	0.00
5	0.00	3.00±0.00	3.00±0.00	2.18^{ab}±0.17	1.18^{a}±0.19	1.01^{a}±0.27	0.32^{a}±0.22	0.00	0.00
6	0.00	3.00±0.00	3.00±0.00	2.34^{ab}±0.24	1.34^{a}±0.24	1.01^{a}±0.27	0.51^{a}±0.23	0.00	0.00
7	0.00	3.00±0.00	3.00±0.00	1.68^{b}±0.24	0.84^{a}±0.19	0.51^{a}±0.25	0.00	0.00	0.00

Score: 0 - NAD, 1 - Mild, 2 - Moderate, 3 - Severe, 4 – Occult blood.

Means bearing different superscripts in the same column differ significantly ($P<0.05$). The method currently being used to discriminate among the means is Tukey's (HSD) procedure.

Effect of drugs on colon wet & dry weight and intestinal damage: Our data showed a clear anti-inflammatory effect of different formulations of drugs administered as a post treatment as this was characterized by a decrease in the colonic damage score, necrotic extension and colonic wet weight. However, the sulfasalazine treated groups showed good improvement of score. Statistically, there was significant ($P<0.05$) difference in the group 1 colon wet weight compared to group 2. Whereas, remaining all other groups does not significantly ($P<0.05$) differ compared to group 1 or 2. There was a significant ($P<0.05$) difference in the intestinal damage noticed in group 2 compared to other groups 3, 4, 5, 6 and 7 whereas, no statistically significant ($P<0.05$) differences were observed among the groups 3, 4, 5, 6 and 7. The results show that, the different formulations of drugs products have equal effect on the re-modulation of intestinal tissue from inflammation and it also posses considerable anti-ulcerative effect(Table 6).

Table 6. Effect of drugs on colon wet weight (Gram) and intestinal damage (Grade)

Group	Mean ± SEM (n = 6)		
	Wet Weight (G)	Dry Weight (G)	Intestinal damage (Grade)
1	0.81^{b}±0.06	0.66^{b}±0.06	0.00
2	1.51^{a}±0.28	1.26^{a}±0.27	4.43^{a}±0.53
3	1.12^{ab}±0.16	0.98^{ab}±0.16	2.29^{b}±0.37
4	1.26^{ab}±0.08	1.03^{ab}±0.07	2.28^{b}±0.37
5	1.13^{ab}±0.17	0.91^{ab}±0.14	2.51^{b}±0.38
6	1.27^{ab}±0.07	1.01^{ab}±0.06	2.51^{b}±0.23
7	1.08^{ab}±0.12	0.88^{ab}±0.11	2.51^{b}±0.38

Intestinal damage score: See Table 2

Means bearing different superscripts in the same column differ significantly ($P<0.05$). The method currently being used to discriminate among the means is Tukey's honestly significant difference (HSD) procedure.

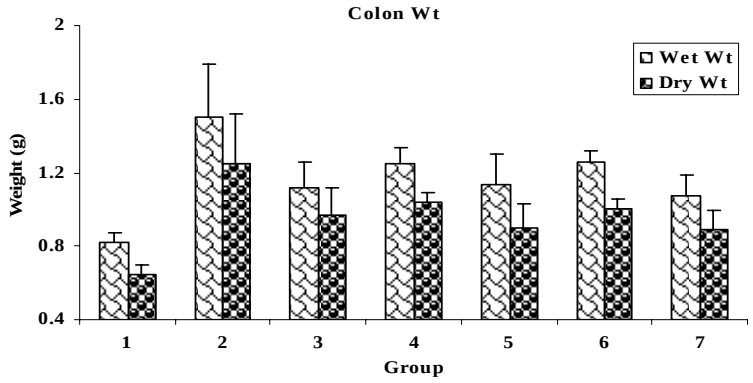

Figure 3. Colon wet weight and dry weight for different groups.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

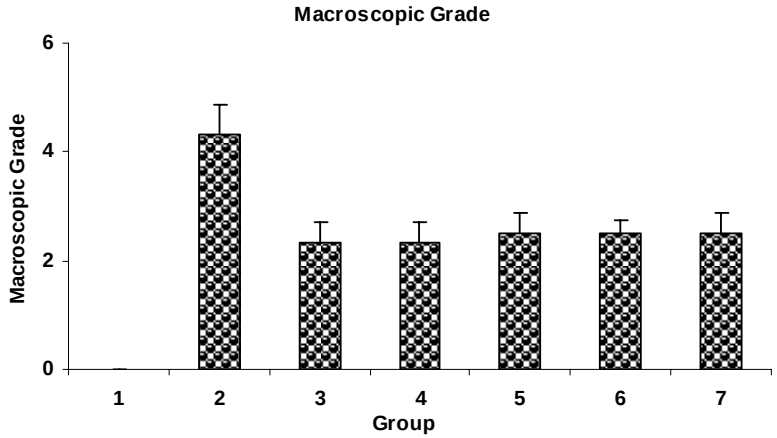

Figure 4. Macroscopic lesion among the groups.

DISCUSSION

Our understanding of the pathogenesis of IBD aims at developing therapies targeted towards controlling the inflammatory cascade which is the result of an aberrant response of the immune system towards luminal antigens.Current therapies for IBD may involve the administration of high dose steroids, immuno-modulators, aminosalicylates and in advanced cases surgery(Fiocchi 2006 and Jonkers et al 2003). Present study was designed to specify the best dose of TNBS for induction of colitis, and to determine ideal reference drugs for the efficacy comparision with test compound as well as treatment of TNBS-induced colitis in animals.

TNBS was precipitated *in vivo* based on our previous trials on Day 1. TNBS, a hapten, elicits its antigenic response when it is bound with high molecular substance like ethanol. An extensive necrosis of the colon is triggered within five days, possibly caused by an oxidative damage. Subsequently an exaggerated innate immune response is stimulated resulting in the generation of cytokines, diarrhea and development of inflammation(Morris et al 1989). Treatment with reference drugs was initiated from Day 3 through Day 9 as this time point is possibly the best to evaluate the anti-inflammatory activity of experimental formulations compared to even the established drugs whose effect tends to be negligible at later stages. The efficacy of the different forms of the current reference drugs formulation was evaluated based on the analysis of body weight, faecal score, feed intake, colon score and colon weights.

The variety of published literature does not suggest that models of colitis are inherently variable or short-lived, yet this is in fact the reality, as emphasized by (Wirtz et al 2007) and noted by (Knollmann et al 2002). Similar experiences have been made by other groups suggesting these problems are common phenomena. Variability resides in the response to the pro-colitic agent and the time course of the resulting pathology, which itself may be divided into 2 stages: First is the acute injury phase due to the actions of TNBS in combination with the mucosal barrier breaker ethanol. In this phase (of 2- 3 days) wound-related events predominate over immunological sequelae, which are likely less usefully targeted by antiinflammatory or immunological agents and of less relevance to IBD. Second is the longer-term immunological sensitization that follows the colonic exposure to TNBS. This therapeutic window appears more relevant to colitis research and anti-IBD therapeutic discovery (Appleyard et al 1995 and Elson et al 1996). Consistent with this findings, we adopted the treatment dosage regimen from Day 2 or Day 3 onwards. Following oral administration three times daily for seven days, a dosing regimen supported by the previous work in our laboratory we found that the different formulations of reference drugs were effective in reducing the intestinal inflammation grade and colon weight compared to TNBS positive control. This is in agreement with the findings of (Peys et al 2007 and Selvam et al 2009) who showed similar anti-inflammatory effects of different doses of reference drugs suggesting that the drug attenuates colitis by acting on inflammatory cytokines. In the colitis group, significant difference in body

gain was observed among the groups compared to TNBS treated group. When testing the effect of reference drugs upon TNBS-induced colitis, (Selvam et al 2009) found lower macroscopic scores and lower TNF-alpha, IL1β, IL6 and IFNγ on the 11th day following colitis induction. In another experiment, (Videla et al 2001) used inulin, a prebiotic consisting of 15 - 40 % polysaccharide chains. Supplementation was provided intragastrically (400 mg/day) in a model with dextran sulfate sodium induced colitis. The area of inflammation was reduced (according to microscopic scores), as was the MPO activity and the release of inflammatory mediators (thromboxane B_2, leukotriene B_4 and prostaglandin E_2). In spite of the reduced number of polysaccharide chains, inulin reduced the inflammatory response in animals with dextran sulfate sodium induced colitis on the fifth day of the experiment.

Unlike sulfasalazine, all other reference drugs used in this study had a significant effect on anorexia and weight loss. In TNBS-induced colitis as well as in human IBD, anorexia occurs as a consequence of actions on the hypothalamus of systemically elevated IL-1β and other factor (El-Haj et al 2002 and Mchug et al 1994). Thus a greater food intake as detected in several reference drugs treated groups may be interpreted as reflecting a lowering of the levels of these mediators secondary to prevention of colitic damage which concurs the findings of (Jaleh Varshosaz et al 2012). According to this finding, we also observed significant improvement in ulcerative colitis as a therapeutic treatment with sulfasalazine. The results of feed intake, colonic damage score and colon wet and dry weight shows that, the different formulations of referene drugs have equal effect on the re-modulation of intestinal tissue from inflammation and it also posses considerable anti-ulcerative effect which concurs the results of (Motavallian et al 2012). This finding showed that there were no formulation dependent effects. According to this finding, we also observed significant improvement in ulcerative colitis as a therapeutic treatment with sulfasalazine.

In conclusion, in the present study, we have demonstrated that different reference drugs influences the healing processes of intestinal mucus membrane and smooth muscle tissue and responsiveness to an inflammatory stimulus. These data support the hypothesis that the different formulations of reference drugs have same ameliorative effect on inflammation. The results were comparable to positive drug control sulfasalazine treated groups as sulfasalazine is gold standard therapy for intestinal inflammatory bowel disease.

ACKNOWLEDGEMENTS

The author thanks to the university for providing all the resourses and help provided for the conduct of the study.

REFERENCES

Ahimou F, Jacques P and Deleu M. (2000). Surfactin and iturin A effects on *Bacillus subtilis* surface hydrophobicity. Enzyme and Microbial Technology. 27: 749 - 54.

Appleyard CB and Wallace JL. (1995). Reactivation of hapten-induced colitis and its prevention by anti-inflammatory drugs. American Journal of Physiology. 269: G119 – G125.

Bais HP, Fall R and Vivanco JM. (2004). Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis roots* by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiology. 134: 307 - 19.

Cavaglieri L, Orlando J, Rodríguez MI, Chulze S and Etcheverry M. (2005). Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides in vitro* and at the maize root level. Research in Microbiology. 156: 748 - 54.

Elson CO, Beagley KW, Sharmanov AT, Fujihashi K, Kiyono H, Tennyson GS, Cong Y, Black CA, Ridwan BW and McGhee JR. (1996). Hapten-induced model of murine inflammatory bowel disease: mucosal immune responses and protection by tolerance. Journal of Immunology. 157: 2174 – 85.

El-Haj T, Poole S, Farthing M J and Ballinger AB. (2002). Anorexia in a rat model of colitis: interaction of interleukin-1 and hypothalamic serotonin. Brain Research. 927: 1 – 7.

Fiocchi C. (2006). Probiotics in inflammatory bowel disease: yet another mechanism of action? Gastroenterology. 131: 2009- 2012.

Fiorini G, Cimminiello C, Chianese R, Visconti GP, Cova D, Uberti D and Gibelli A. (1985). *Bacillus subtilis* selectively stimulates the synthesis of membrane bound and secreted IgA. Chemioterapia. 4: 310 – 312. Cross reference from Teo AY and Tan HM. 2006. Effect of *Bacillus subtilis* DRUGS (CloSTAT) on Broilers Infected with a Pathogenic Strain of *Escherichia coli*. Journal of Applied Poultry Research. 15: 229 – 235.

Henriques AO, Melsen LR and Moran CP Jr. (1998). Involvement of superoxide dismutase in spore coat assembly in *Bacillus subtilis*. Journal of Bacteriology. 180: 2285 - 2291.

Jaleh Varshosaz, Jaber Emami, Naser Tavakoli, Mohsen Minaiyan, Nakisa Rahmani, Farid Dorkoosh and Parvin Mahzouni. (2012). Pectin Film Coated Pellets for Colon-targeted Delivery of Budesonide: *In-vitro/In-vivo* Evaluation in Induced Ulcerative Colitis in Rat**.** Iranian Journal of Pharmaceutical Research, 11 (3): 733-745.

Jonkers D and Stockbrügger R. (2003). Probiotics and inflammatory bowel disease. Journal of the Royal Society of Medicine. 96: 167 - 171.

Knollmann FD, Dietrich T, Bleckmann T, Böck J, Mäurer J, Radtke C and Felix R. (2002). Magnetic resonance imaging of inflammatory bowel disease: evaluation in a rabbit model. Journal of Magnetic Resonance Imaging. 15: 165 – 73.

Leifert C, Li H, Chidburee S, Hampson S, Workman S, Sigee D, Epton HA and Harbour A. (1995). Antibiotic production and biocontrol activity by *Bacillus subtilis* CL27 and *Bacillus pumilus* CL45. Journal of Applied Bacteriology.78: 97 - 108.

Liu Y, Chen Z, Ng TB, Zhang J, Zhou M, Song F, Lu F and Liu Y. (2007). Bacisubin, an antifungal protein with ribonuclease and hemagglutinating activities from *Bacillus subtilis* strain B-916. Peptides. 28: 553 - 59.

McHugh KJ, Collins SM and Weingarten HP. (1994). Central interleukin-1 receptors contribute to suppression of feeding after acute colitis in the rat. American Journal of Physiology. 266: R1659 – R1663.

Mohammadipour M, Mousivand M, Salehi Jouzani G and Abbasalizadeh S. (2009). Molecular and biochemical characterization of Iranian surfactin-producing *Bacillus subtilis* isolates and evaluation of their biocontrol potential against *Aspergillus flavus* and *Colletotrichum gloeosporioides*. Canadian Journal of Microbiology. 55: 395 - 404.

Motavallian-Naeini A, Andalib S, Rabbani M, Mahzouni P, Afsharipour M and Minaiyan M, (2012). Validation and optimization of experimental colitis induction in ratsusing 2, 4, 6-trinitrobenzene sulfonic acid. Research in Pharmaceutical Sciences. 7(3): 159-169.

Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR and Wallace JL. (1989). Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology. 96: 795 – 803.

Muscettola M, Grasso G, Blach-Olszewska Z, Migliaccio P, Borghesi-Nicoletti C, Giarratana M and Gallo VC. (1992). Effect of *Bacillus subtilis* spores on interferon production. Pharmacology Research. 26: 176 – 177.

Ngugia HK, Dedejb S, Delaplaneb KS, Savellea AT and Scherma H. (2005). Effect of Xower-applied *Serenade biofungicide* (*Bacillus subtilis*) on pollination-related variables in rabbiteye blueberry. Biological Control. 33: 32 – 38.

Peys E, Varghese J, Suresh P, Vandenkerckhove J, Van hemel J, Ramchand CN and Sas B. (2007). Effect of *Bacillus subtilis* 'PB6' (ATCC – PTA 6737) on *Clostridium difficile* associated diarrhea (CDAD) and inflammatory bowel disease (IBD) in animal models. American Journal of Infectious Diseases. 3: 253 – 365.

Sands BE. (2000). Therapy of inflammatory bowel disease. Gastroenterology. 118: S68-82. Review.

Selvam R, Kavitha P, Ravichandran M, Sas B and Ramchand CN. (2009). Effect of *Bacillus subtilis* PB6, a natural probiotic on colon mucosal inflammation and plasma cytokines levels in inflammatory bowel disease. Indian Journal of Biochemistry and Biophysics. 46: 79 – 85.

Sharma N and Sharma S. (2008). Control of foliar diseases of mustard by *Bacillus* from reclaimed soil. Microbiology Research. 163: 408 - 13.

Stephen J. (2001). Pathogenesis of infectious diarrhea. The Canadian Journal of Gastroenterology. 15: 669 - 683.

Teo AY and Tan HM. (2005). Inhibition of *Clostridium perfringens* by a novel strain of *Bacillus subtilis* isolated from the gastrointestinal tracts of healthy chickens. Applied and Environmental Microbiology. 71: 4185 – 4190.

Teo AY and Tan HM. (2003). The effect of heat, pH and bile salts on a new strain of *Bacillus subtilis* isolated from the gastrointestinal tract of healthy chicken. Abstr. 12th World Food Science Technology Congress, Chicago, IL. IFST, UK.

Videla S, Vilaseca J, Antolín M, García-Lafuente A, Guarner F, Crespo E, Casalots J, Salas A and Malagelada JR. (2001). Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat. The American Journal of Gastroenterology. 96: 1486 - 1493.

Wirtz S, Neufert C, Weigmann B and Neurath MF. (2007). Chemically-induced mouse models of intestinal inflammation. Natural Protocols. 2: 541 – 46.