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TNBS- INDUCED ILEITIS WITH VISCERAL HYPERSENSITIVITY IN DOGS

Sayyed Aun Muhammad[1], Abdul Shakoor[1,*], Muhammad Ijaz[2], Syed Ehtisham-ul-Haque[1] and Adnan Hassan Tahir[3]

[1]University of Veterinary and Animal Sciences, Lahore, Sub-campus, Jhang-Pakistan; [2]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore-Pakistan; [3]Chengdu University of Traditional Chinese Medicines, Chengdu, China
***Corresponding author's e-mail: abdul.shakoor@uvas.edu.pk**

Sixty clinically healthy dogs were divided into two groups i.e., saline (n=30) and TNBS group (n=30). One mL of ethanol mixed TNBS solution (30 mg TNBS in 30% ethanol) was injected in the ileal wall in each dog of the TNBS group after performing laparotomy. In the saline group, one mL of normal saline was injected in the same way. The severity of the inflammatory response was assessed by macroscopic, microscopic scoring, and plasma level of interleukin (IL-6). Visceral hypersensitivity (VH) in these dogs was reflected by pain response score (PRS) due to colorectal distension (CRD) by inserting colorectal distension device on day 2, 7, 13, 19, and 25. TNBS treated dogs showed transmural pathologic changes as compared to the saline-treated group. A significant increase ($P < 0.05$) in microscopic, macroscopic scores and plasma IL-6 level was observed in the TNBS treated group as compared to the saline group. Dogs showed apparent ileitis from day 2 to 19 and VH was observed from day 7 to 25. The experiment successfully established a reproducible ileitis model in dogs with VH that will help to further study the pathogenesis of IBD and the effect of different therapeutics to treat IBD.
Keywords: IBD, Visceral Hypersensitivity, TNBS, Ileitis Model, and Pain Response Score.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract (GIT) of animals and humans. Ulcerative Colitis (UC) and Crohn's Disease (CD) are the two known forms of IBD. Crohn's Disease is a major form of IBD in humans as well as in dogs. It mainly causes the inflammation of the entire wall of the ileum. Chronic and uncontrolled inflammation of intestinal mucosa is the feature of IBD (Papadakis and Targan, 2000). The diagnosis of IBD can be made by either structural distortion or the presence of an increased number of inflammatory cells in the affected area of the intestine or colon. The difference between IBD and other inflammatory responses is that the inflammation is not down-regulated in IBD and there is chronic activation of the mucosal immune system making the intestines chronically inflamed (Hanauer, 2006). Inflammatory bowel disease is common in dogs and cats and is associated with loss of appetite, decrease intestinal activity, chronic diarrhea, vomiting, and weight loss (Jergens *et al*., 1992). Despite the research of many years in humans and dogs, the exact cause of IBD is unknown. However, like humans, environmental, genetic, and immune system deregulations are thought to be precipitated factors in dogs (Garcia-Sancho *et al.,* 2007). Food allergy, parasitism, imbalance in bacterial activity, and specific breeds are considered as contributory factors for IBD in dogs and cats. Diagnosis usually involves the biopsy of intestines (Choi and Appelman, 2017).

Visceral Hypersensitivity (VH) is one of the important symptoms of CD that presents lifelong relapsing and remitting problems affecting the quality of life. It is considered a major therapeutic challenge for physicians (Aarons, 2013). Visceral Hypersensitivity along with pain is considered as a feature and possible biomarker of IBD (Melchior *et al.,* 2018). In IBD, patients with VH have more likely hood of pain as compare to those which are non-hypersensitive (Azpiroz *et al*., 2017). Regulation of GIT immunity against different enteric antigens (microorganisms) may result in provoking VH (Adam *et al.,* 2013).

An understandable mechanism for the occurrence of VH is still unknown, however, certain mechanisms have been given by certain researchers such as inflammation or sensitization after injury and after enteric infections (Zhou and Verne, 2011). Because the spontaneous development of CD-like lesions in animals is rarely found, so most researchers used the colitis model for CD research (Deiteren *et al.,* 2015). VH is a topic of passionate research (Kanazawa *et al.,* 2011). Because of certain factors e.g. locations, timing, and presence of microflora, the colitis models could not prove precise. Gadaleta *et al.* (2017) established the colitis model in rats by intra-rectally passing TNBS in 50% ethanol and develop IBD-like symptoms for two weeks with mortality of rats. Cervi *et al.* (2017) develop the DSS colitis model in mice by giving DSS with drinking water, but an important feature of CD-like transmural inflammation and ulceration was not studied. Merritt *et al*. (2002) used intra-intestinal instillation of TNBS

Ethanol via hypodermic needle and found no ileal lesions in some pigs due to no confinement of chemical in 10-15 cm long length of the ileum.

Interleukin (IL-6) plays a key role in many biological activities e.g., inflammation, immune regulation, and oncogenesis etc. Th17 cell formation from naïve T cells together with TGF-β is carried out by IL-6 (Kimura and Kishimoto, 2010). A complex is formed between IL-6 and soluble IL-6R. This complex can bind to those cells having no IL-6 receptors. This prevents the T-cell apoptosis of mucosa and lamina propria and contributes to inflammatory conditions like CD (Atreya *et al*., 2000).

Adam *et al*. (2006) and Adam *et al*. (2013) studied the IL-6 level in TNBS induced rat colitis model and observed that the level of IL-6 was higher in the acute phase of colitis and it was also higher in the initial chronic phase of colitis and decreased to the background level at termination phase of inflammation.

The present study has been designed to develop TNBS induced ileitis with VH in dogs. To evaluate the severity of resulting ileal inflammation, macroscopic, and microscopic damage scoring was measured. Pain behavior scoring in response to CRD was done to assess VH. The dynamic profile of cytokine (IL-6) was determined. Based on the results, a successful ileitis model with VH was developed, which could be used to study the effect of different therapeutics to treat visceral hypersensitivity.

MATERIALS AND METHODS

***Preparation of dogs for experiments*:** All the dogs were physically examined to assess the health status. The same nutritional plan was offered to all animals. Deworming of each dog was done a week prior to the start of the trial. The experiment was approved (vide letter no. DR/618 dated 20/6/18) by the Ethical Committee for Experimental Animals, University of Veterinary and Animal Sciences, Lahore-Pakistan. Guidelines issued by the international association for the study of pain were followed during the whole experiment.

***TNBS dosage for induction of ileitis*:** For this study, fifteen clinically healthy stray dogs of either sex were used. Five random groups were made i.e., Saline, Ethanol (30%), TNBS 20 (20mg in 30% ethanol), TNBS 30 (30mg in 30% ethanol), and TNBS 40 (40mg in 30% ethanol) having three dogs in each group. To overcome any anesthetic complication, the water and feed of dogs were restricted 12 hours prior to the experiment. Dogs were pre-anesthetized with atropine sulphate (Atrostar, Star lab. Pakistan) @ 0.04mg/kg I.M and Xylazine HCl (Xylaz, Forvet) @ 0.2mg/kg IV. The animals were anesthetized by intravenous (IV) injection of Ketamine HCl (Ketarol) @ 5mg/kg BW. Endotracheal intubation was done to prevent aspiration of gastric secretions. A 5-cm long middle midline ventral abdominal incision was made to approach and exteriorize the distal part of the ileum. In Saline Group, 1mL of normal saline was injected intramurally. Four points of ileum were used starting 5 cm away from the ileocecal junction. A 30 gauge needle was used. In the Ethanol group, 1mL of 30% ethanol was injected intramurally in 4 points of ileum. While other 3 groups were injected with 1ml of 30% ethanol with different concentrations of TNBS (Sigma Aldrich Company, USA). After placing the intestine back, the abdominal wall and skin were approximated by an interrupted suture pattern. The dogs were kept isolated for 1-2 hours for anesthesia recovery. Povidone-iodine 1% was applied daily on surgical wounds until complete healing. All animals from each group were passed through the same surgical procedure to expose the ileum on Day 5. The 4-cm piece of ileum was resected leaving 5 cm from the junction of ileum and caecum.

***Model Experiment*:** Experimental setup is shown in Figure 1. Sixty clinically healthy stray dogs (30 male and 30 female, about one-year-old) were included in this trial. A thorough examination of stray dogs was done to assess health status. Two random groups were used i.e., TNBS group (n=30) and the saline group (n=30) with 30. Dogs were pre-surgically prepared and anesthetized by using the same protocol used in the pre-experimental trial for dose determination of TNBS as demonstrated by Tahir *et al.* (2015). A 5-cm long middle midline ventral abdominal incision was given to approach and exteriorized the distal part of the ileum. One mL injection of TNBS-Eth. Solution (30 mg TNBS in 30% ethanol) was given intramurally in dogs of TNBS group. Four points of ileum were used starting 5 cm away from the ileocecal junction. The intramural injection will increase the contact time of TNBS with mucosal cells of ileum to induce transient ileitis (Tahir *et al*., 2015). One ml of saline was administered intramurally in each dog of the saline group in the same manner. After placing the intestine back, suturing of the abdominal wall and skin was done using a simple interrupted suture pattern. The dogs were kept isolated for anesthesia recovery for 1-2 hours. Povidone-iodine 1% was applied daily on surgical wounds until complete healing. Weights of dogs were taken on day 0, 2, 7, 13, 19, and 25. Six dogs from both groups were passed through the same surgical procedure at days 2, 7, 13, 19, and 25 to expose the ileum immediately after doing Pain Response Scoring (PRS) in response to Colorectal Distension Testing. A 4-cm piece of ileum was resected leaving 5 cm from the junction of ileum and caecum. This resected piece of ileum was washed with PBS and longitudinal cuts were made. The macroscopic changes in the mucosa were observed. 1.5×1.5 cm sized tissue samples were stored in 10% formalin for histopathological observation. The end-to-end anastomosis of ileum was performed immediately.

***Visceral Hypersensitivity reflection by Colorectal Distension (CRD) Testing*:** Visceral Hypersensitivity in dogs of both groups was measured by PRS. The pain response score was assessed by monitoring Behavioral Pain Responses to Colorectal Distension. Pain behavioral scale ranging from

0, 1, 2, 3 and 4 was used for this study. A manually operated Colorectal distension device was made by modification of the sphygmomanometer. After lubrication, the polyethylene balloon (12 cm) part of the device was inserted from anus to the distal part of colon about 10 cm from the anus of the dog. After 15 minutes of acclimatization, inflammation of the balloon was done with the pump. Balloon pressure was counted with the sphygmomanometer. Balloon pressure was increased from 20 to 120 mmHg stage by stage and lasted for 6 seconds at each stage. The pain response score was recorded during each stage. Two observers used to observe the PRS who were blind to the experimental conditions. The pain response score was measured three times and was averaged (Tahir *et al.*, 2015).

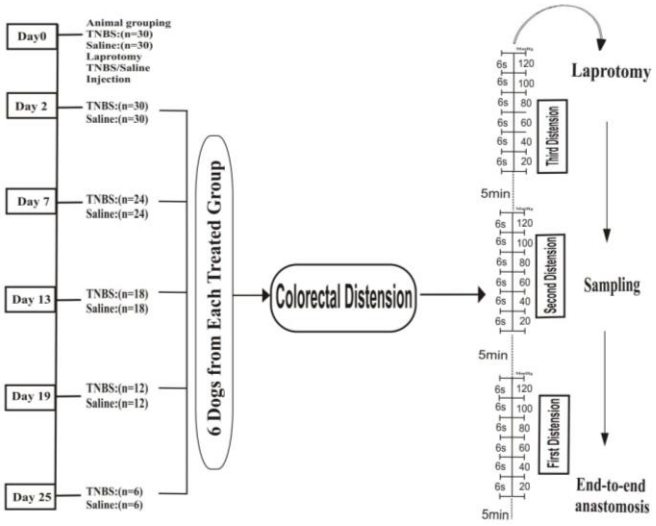

Figure 1. The model experiment design.

***Gross and Histological observations*:** Macroscopic lesions were scored separately and were assessed by gross observation of ileal wall strictures, adhesions of ileum with neighboring tissues, ulcers on the mucosal membrane, and thickness of the ileal wall (Tahir *et al.*, 2015). Processing of samples was done for histological studies. Three series of tissue sections with 5µm thickness were obtained. These sections were stained with hematoxylin-eosin (HE) (Tahir *et al.*, 2015). Microscopic scoring was done by two pathologists separately. The scoring criteria were fixed based on tissue edema, infiltration of inflammatory cells, ulcerations and villus fusion.

***Plasma concentration of Interleukin-6 (IL-6)*:** Blood samples were taken from each group of dogs on days 2, 7, 13, 19 and 21 after the colorectal distension test. The plasma level of IL-6 concentrations was measured using a commercially available ELISA kit (mlbio canine interleukin 6, China) following the manufacturer's instructions.

***Statistical Analysis*:** For each experiment, Mean ± SD was used for the expression of experimental data. Data were analyzed using SPSS software. One way ANOVA (Parametric or Nonparametric) was used in order to quantify the Dose of TNBS in 30% ethanol. Mann–Whitney U Test was performed for analysis of scoring grade of Visceral Pain in response to Colorectal Distension. Data having P value less than 0.05 was were considered significant.

RESULTS

***Determination of ileitis induction dosage of TNBS*:** Increasing concentration of TNBS (0 mg to 40 mg) in 30% ethanol increased the severity of inflammation of ileum. Gross scores recorded in TNBS 30 group (30 mg of TNBS in 30 % Eth.) were 6.78±0.19 and the microscopic score was 6.89±0.19. Both were found higher ($P<0.05$) than 30% ethanol group (4.00±0.33, 4.11±0.19) and TNBS 20 group (20 mg TNBS in 30% ethanol) 4.78±0.51 and 5.11±0.51 respectively, but were lower than TNBS 40 group in which 40 mg TNBS was used in 30% ethanol. The values obtained for gross and microscopic scores were 8.00 ±0.33 and 8.56 ±0.20, respectively. Therefore, a dose of 30 mg TNBS in 30% ethanol was chosen for induction of ileitis in this experiment because it induced moderate ileitis.

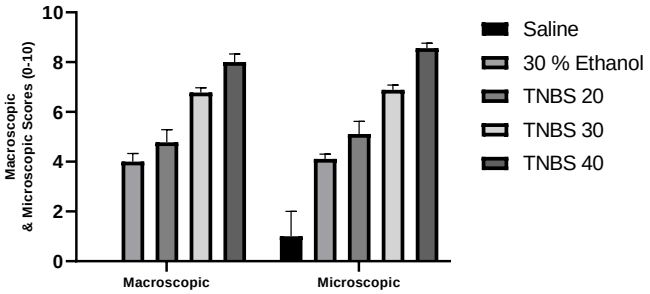

Figure 2. Macroscopic and Microscopic Scores (0-10) of 5 different groups of treatments measured on day 5.

Effect of TNBS induced ileitis on bodyweight: In Saline group and TNBS group, dogs showed an average decrease in the bodyweight with -2.13 kg% and -4.12 kg% respectively at day 2 (p=.007). The bodyweight of the TNBS-treated Group was also decreased (-2.45 kg%) at day 7 whereas for the saline group it increased by 3.54% with a p-value of .000. On day 13, there was an increase in the average body weights of dogs in both groups (4.98±1.60% and 2.71±.71) with P=.010. At day 19, the body weights of both groups were increased by 6.27±1.18% for saline and 3.83±.66% for TNBS with p=.001. Similarly, at day 25, the increase in body weight in the Saline group was observed by 9.33±1.32% and for TNBS group was 5.39±.47% with P=.000. Comparing with the saline-treated group, the body weight of dogs in the TNBS treated group was found lower (*P*=.007, .000, .010, .001, .000) at days 2, 7 and 13, 19 and 25.

Table 1. Criteria for the scoring morphological damage of ilium.

Gross Changes		Microscopic Changes	
Adhesions	None (0) Minimum (1) Multiple bowel loops involved (2)	**Edema**	None (0) Mild (1) Moderate (2) Severe (3)
Mucosal Hyperemia	Normal (0) Mild (1) Moderate (2) Severe (3)	**Inflammatory Cells**	None (0) Mild (1) Moderate (2) Severe (3)
Ulcers	None (0) 1 ulcer < 2 cm length (1) 2 ulcers <2 cm (2) More ulcers (3)	**Ulceration**	None (0) Moderate ulceration (1) Severe ulceration (2)
Wall Thickness	Normal (0) 50% increase (1) 100% increase (2)	**Villus Fusion**	Normal (0) 25% fusion (1) More than 25% fusion (2)
Maximum scores for Gross and Microscopic damage			10

Macroscopic and Microscopic changes of TNBS-treated ileum: At day 2, macroscopically, the ileal mucosa showed congestion, edema, wall thickness and tissue necrosis in TNBS treated group. While at day 7, wall thickness, tissue necrosis and congestion of ileal tissues were also found. On Day 13, there was mild congestion of mucosa and wall thickening (Fig. 3). Adjacent organs including jejunum, colon, and mesentery were not having any lesion. Gross lesions scores recorded in the TNBS-treated dogs at days 2, 7, and 13 were 8.11±0.40, 6.00±0.52 and 4.5±0.46, which are severe in saline group (2.11±0.27, 1.89±0.40 and 1.61±0.33). However, macroscopic lesions were not found different (P >0.05) at days 19 and 25 between TNBS and Saline groups (Fig. 3).

At day 2, microscopic changes observed showed minute infiltrated inflammatory cells in the Saline group, but on days 7 to 25, no infiltration of inflammatory cells was observed. While ileal wall of TNBS treated group presented an inflammatory cell infiltration and ulceration of sub-mucosa and muscular layer at day 2 and 7. Extensive inflammatory cell infiltration was observed at day 13, and a moderate level of infiltration was observed at day 19. Microscopic damage scores in the TNBS-treated group at day 2, 7, 13, and 19 were 8.67±.30, 5.83±0.55, 4.39±0.39 and 1.89±.27 which are severe (P<0.05) than those of the saline group (2.50±0.46, 2.22±0.34, 2.00±.37 and 1.50±0.35). However, no significant difference (P>0.05) was observed in the microscopic damage between the two groups on day 25 (Fig. 4).

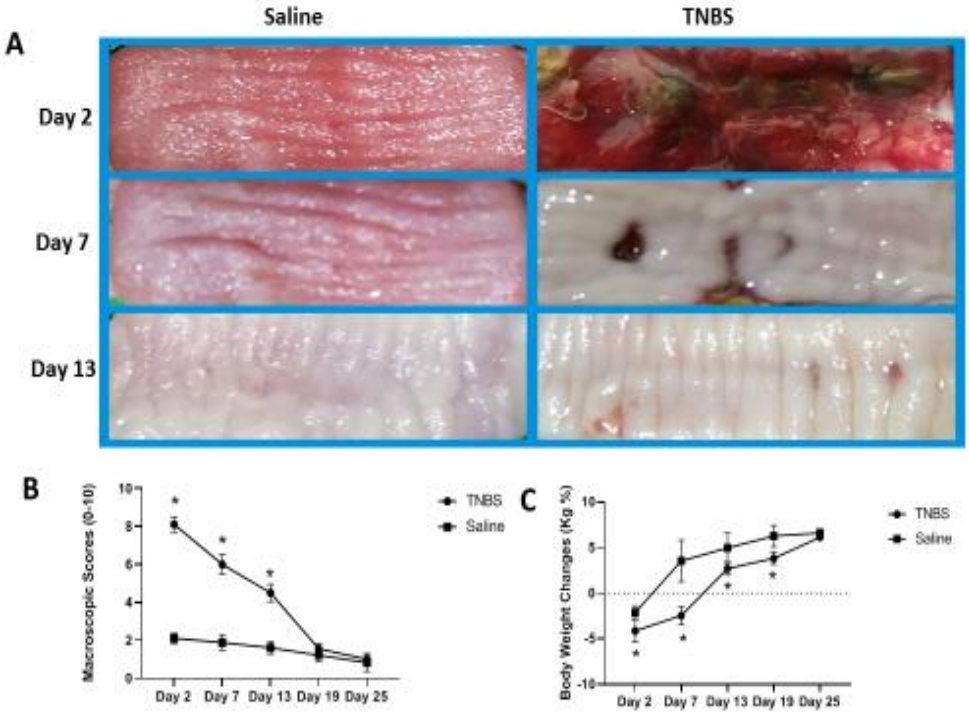

Figure 3. Gross Lesions Variations between Saline and TNBS (a), macroscopic lesions scores (b) changes in body weight (c) after TNBS and Saline treatment in dogs. There was sever inflammation of ileum, ulcer formation from day 2 to 7 in TNBS group. The severity of damage was decreased on day 13.

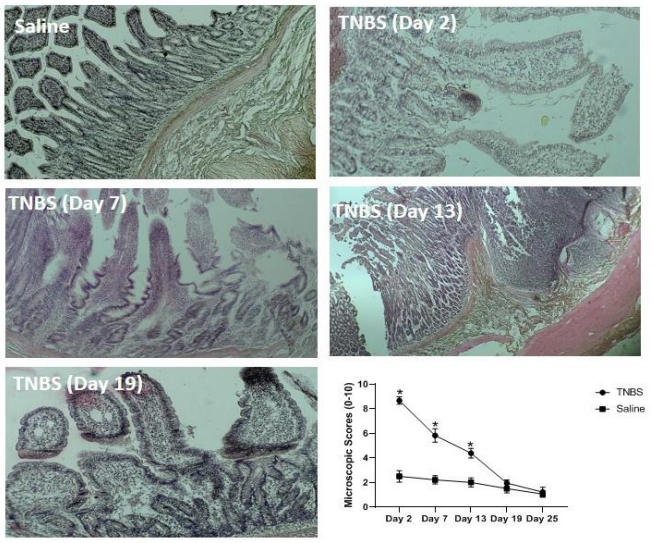

Figure 4. Microscopic changes and microscopic lesions score of the TNBS-treated and Saline treated ileum in dogs. In the saline group, No significant inflammation and microscopic changes. In TNBS treated group, infiltration of the inflammatory cells and ulceration of mucosal and sub-mucosal layers was observed on days 2 to 7. Inflammatory cells infiltration is extensive at day 13, and less severe on day 19.

Pain Response Score (PRS) after Colorectal Distension: Pain response score was measured after graded pressure of balloon distension in colo-rectum at days 2, 7, 13, 19 and 25. A pressure of 20, 40, 60, 80, 100 and 120 mmHg was used. Pain response scoring was made after observing the clinical signs like agitation, change of position, head backing towards abdomen and vocalization in the response of balloon distension pressure in the colon. On day 2, no difference in PRS between the saline treated group and TNBS group was observed. Pain response score was higher (P<0.05) in TNBS treated dogs than saline-treated dogs at different pressure levels at days 7 to 25 excluding 20 mmHg and 40 mmHg pressure. PRS in the TNBS group was increased (P<0.05) at 60, 80, 100 and 120 mmHg on day 7. At day 13 and 19, PRS in the TNBS-treated dogs was increased (P<0.05) at 60 mmHg pressure and achieved peak level (P<0.01) at 80, 100 and 120 mmHg. At day 25, PRS in the TNBS group was increased (P<0.05) at 60 and 80 mmHg, and further increased (P<0.01) with 100 and 120 mmHg (Fig. 5).

Plasma level of IL-6: Plasma level of IL-6 was found almost the same (48.10± 5.59 pg/ml to 51.93± 7.57 pg/ml) from day 2 to 25 in the saline group. Significant elevation in the plasma concentration of IL-6 was found in TNBS group on day 2, 7 and 13 (447.99±6.15, 280.38±10.28 and 123.76±5.77 pg/ml) but this level was decreased at day 19 and 25 (57.96±5.43, 58.83±7.78) (fig. 6)

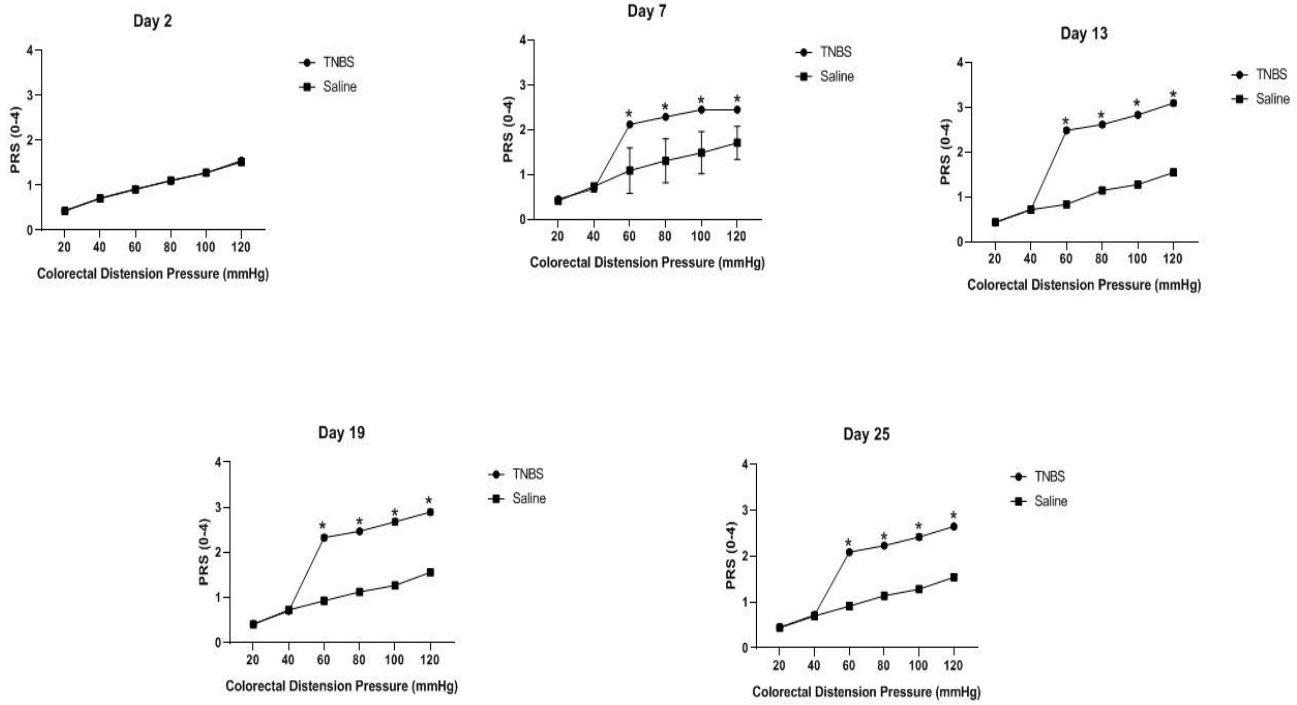

Figure 5. Pain response score (PRS) in dogs in response to colorectal distension (CRD) in TNBS treated groups at days 2, 7, 13, 19 and 25 after treatments. The mean values show a significant difference compared with the Saline (control) group.

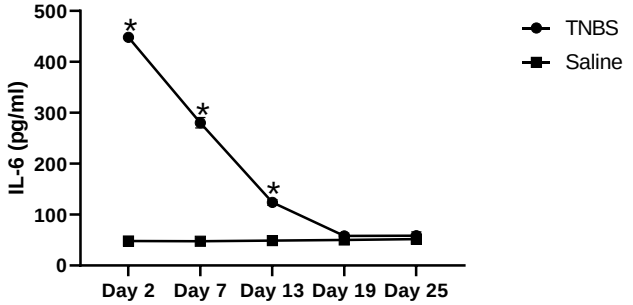

Figure 6. Measurement of IL-6 (pg/ml) in plasma after administration of TNBS shows a significant difference at day 2,7,13 while decreased at day 19 and 25.

DISCUSSION

To observe the pathogenesis of acute and chronic colitis, colitis model has been established using colorectal instillation of TNBS-Ethanol solution (Adam *et al*., 2006; Adam *et al*., 2013). Cervi *et al*. (2017) induced colitis in mice by oral feeding of 5% DSS with drinking water. Some researchers also used TNBS induced ileitis model to study the pathogenesis of acute and chronic ileitis in different lab animals. Moore *et al*. (2002) and Stewart *et al*. (2003) developed TNBS ileitis in guinea pigs to study the hyper-excitability response. Tahir *et al*. (2015) and Wan *et al*. (2017) produced TNBS ileitis in goats to study the VH. Most of the findings of these researches showed a difference in response from TNBS induced ileitis to that colitis. There is a difference in routes of administration and doses of TNBS in different studies. Different protocols have been followed by different researchers. Moreels *et al*. (2001) used TNBS 85 mg/kg in 40% ethanol and found severe ileitis in rats after 36 hours which remained for 7 days. Rats showed severe changes occurring in the concentration of MPO and Macroscopic and Microscopic structural changes. In another study by O'Hara *et al*. (2007), TNBS in 30% ethanol was given @ dose of 60 mg/kg into the lumen of ileum of Guinea pigs. Inflammation was observed at 3rd day of treatment. Nurgali *et al*. (2007) used TNBS in guinea pigs with a dose of 30 mg/kg in 30% ethanol and found the inflammatory response up to day 7 of treatment. Merritt *et al*. (2002) used the injection of TNBS-ethanol solution in pig's ileum, and found ileitis for up to 7 days. These studies developed transient ileitis or colitis by the use of TNBS-ethanol solution in different animals. TNBS solutions with a dose-ranging from 10 from 40 mg/rat or 30 to 150 mg/kg body weight in 30 to 50% ethanol used into the lumen of colon or ileum to induce colitis or ileitis in most of the studies performed on rats. Tahir *et al*. (2015) and Wan *et al*. (2017) used 30 mg TNBS in 40% solution of ethanol to induce apparent inflammation and transmural lesions of ileum in goats. These outcomes of different researches revealed that independent use of 30mg TNBS or 50% ethanol alone resulted in mild colitis for less than 7 days while a combination TNBS (10 to 30mg) with 30 to 50% ethanol solution can result in mild to severe colitis or ileitis, which can continue for 21 days in rats (Boughton-Smith*et al*, 1988; Morris *et al*., 1989). In present study, different concentrations of TNBS were used with 30% ethanol intramurally in dogs which resulted from in mild to severe ileitis. From this, it is believed that use of TNBS and ethanol solution on the intestinal tissue established a better inflammatory response. TNBS when applied on the intestinal tissue turns as hapten after binding with intestinal tissue proteins. It provokes inflammatory and immune responses and results in VH (Adam *et al*., 2006), whereas ethanol may contribute to tissue diffusion of TNBS and the development of acute inflammation. Many other factors can influence the effect of TNBS-ethanol solution for the induction of transient inflammation of ileum. Mucous secretion, intestinal emptying, and intestinal peristalsis movement may decrease the time of contact of TNBS with mucosa affecting the severity and duration of ileitis (Merritt *et al*., 2002). A modified protocol was applied by Czaja *et al*. (2005) who injected 4% formaldehyde in the ileal wall of pigs on multiple sites and observed transient inflammation of ileum at day 3.

Use of intramural injection of TNBS-Eth. solution for induction of ileitis has not been reported in dogs. Shibata *et al*.(1993) used TNBS solution in lumen of ileum but not in the wall. In the present study, 30 mg TNBS mixed in 30% ethanol was injected in the ileal wall that produced moderate ileitis in dogs. Dogs presented the clinical signs of weight loss and diarrhea. Certain histo-pathological apparent changes including vascular congestion, mucosal ulceration, infiltration of neutrophils and lymphocytes were also observed. These symptoms and pathological changes have a quite resemblance with IBD. In present study, TNBS-induced ileitis was observed on day 2, which continued till day 19 and diminished on day 25.

The most annoying symptoms observed in IBD are abdominal pain and discomfort present during acute as well as the remitting phase of the disease (De Schepper *et al*., 2008; Vermeulen *et al*., 2014). Adam *et al*. (2006) applied TNBS in Lewis rats through colorectal infusion, found VH at days 28 to 42. In a study by Zhou *et al*. (2008) 20mg TNBS in 50% ethanol was used in the colon of SD rats, VH was observed at days 2 to 28. Paiotti *et al*. (2012) used intra luminal administration of TNBS in mice. They observed VH at days 7 to 14. Shah *et al*. (2016) gave TNBS-ethanol solution into the lumen of rat's ileum and observed ileitis at day 3. They observed VH from 7 to 21 days after treatment. The difference in onset time and duration of VH mentioned above may be due to the difference in location of inflammation and species. In our study, TNBS-Eth. solution was used by intramural injection in ileum of dogs, a significant Visceral Hypersensitivity was observed at day 7 and it continued to day

25. These finding are similar to the results of Tahir *et al*., (2015). They observed VH in goats which was continued up to day 28. As our experiment was conducted up to day 25; how long the VH exists, needs further confirmation.

The causes of development of VH are unknown, it is observed that pro-inflammatory cytokines such as IL-6 seem to be provoking the VH. Adam *et al*. (2013) induced Visceral Hypersensitivity in Lewis rats and observed an increased level of IL-6 in the acute phase of TNBS colitis. VH remained persistent even in the chronic phase of colitis in which IL-6 level was decreased to normal. Adam *et al*. (2006) found the association of IL-6 with increased VH and severity of colitis during acute phase of inflammation up to day 14 in Lewis rats. But after 28 days, VH level was increased even the IL-6 level was normal. In our study, mark increase in IL-6 level was observed from day 2 to 13 during the acute phase of ileitis. VH was increased during this phase. But after day 19, the IL-6 level get normal but VH continued to remain persistent up to day 25.

***Conclusions*:** The main accomplishment of this study was to develop a TNBS induced ileitis with hypersensitivity in dogs. The use of large animal subjects such as dogs as compare to lab animals allowed for gross mucosal changes of ileum and obvious pain response scoring. Different doses of TNBS/Ethanol Injection in the wall of ileum of dogs caused dose-dependent severity of ileitis. A dose of 30mg TNBS mixed in 30 % ethanol injected into the terminal ileal wall of dogs resulted into apparent inflammation and transmural lesions, which are morphologically similar to inflammatory bowel disease. VH was evident from day 7 after TNBS/ET injection into the wall of ileum and continued to day 25. An increase level of IL-6 was observed during the acute phase of ileitis from day 2 to 13 of trial and it was decreased to normal after day 19. The experiment successfully constructed a reproducible ileitis and VH in dogs, which is useful to study the pathogenesis of IBD in dogs and for evaluating the efficacy of new therapeutic regimens to treat IBD in dogs and humans as well because of anatomical similarities of dogs with human beings.

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