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ATTENUATION OF VISCERAL HYPERSENSITIVITY DUE TO TNBS-INDUCED ILEITIS BY ELECTROACUPUNCTURE, *Nigella sativa*, *Eugenia caryophyllata* OIL AND SURGICAL RESECTION OF ILEUM IN DOGS

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The study aimed to document the therapeutic effect of application of Electroacupuncture (EA), oral administration of *Nigella sativa* (NS), *Eugenia caryophyllata* (EU) oils and surgical resection of ileum on TNBS induced ileitis with visceral hypersensitivity (VH) in dogs.I ml solution containing 30 mg of TNBS in 30 % ethanol was injected into ileal wall of dogs of TNBS group, TNBS+ EA group, TNBS + NS group and TNBS + EU group. In saline group, an equal quantity of saline was injected instead of TNBS solution. Dogs in the EA group were treated with EA at day 6, 9, 12, 15, 18 and 21 after TNBS treatment while dogs in TNBS+ NS and TNBS+ EU group were orally administered with NS oil and EU oil @2.5 ml /kg on day 6, 9, 12, 15, 18 and 21. VH in dogs of all groups was reflected by Pain Response Score (PRS).PRS was assessed by monitoring Behavioral Pain Responses to Colorectal Distension at day 6, 9, 12, 15, 18 and21 in all groups.Blood samples were taken from each group of dogs on day 6, 9, 15, 18 and 21 after colorectal distension test to measure the Plasma IL-6 Concentrations. Dogs were passed through laparotomy procedure to get ileal tissue samples from each group. Macroscopic and microscopic scoring of each sample was done. Three dogs from saline group (n=3) and three dogs from TNBS group (n=3) were further tested to see the effect of surgical resection of ileum on VH. After induction of ileitis with TNBS/Ethanol dogs exhibited the signs of Vomiting, diarrhea, and decreased body weight. Macroscopic and Microscopic scoring of ileal tissue was increased, plasma concentration of IL-6 was increased and Pain response score in response to colorectal distension was also increased. EA application, oral administration of NSand EU oil relieved the VH by reducing the Pain behavior response, Microscopic and Macroscopic scoring and plasma concentration of IL-6. Surgical resection of effected part of ileum also relieved the VH.

Keywords: Visceral hypersensitivity, TNBS-induced ileitis, Electroacupuncture, *Nigella sativa*, *Eugenia caryophyllata* and Pain behavior response.

INTRODUCTION

Inflammatory Bowel Disease (IBD) is a group of chronic inflammatory diseases of the gastrointestinal tract (GIT) in animals and humans. Crohn's Disease (CD) and Ulcerative Colitis (UC) are the two forms of IBD. CD 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. The continuous and retained occurrence of Visceral Hypersensitivity (VH) is an important concern of Inflammatory Bowel Disease (IBD). Due to lack of understanding of VH, no optimal treatment has been found effective. At present the medicines being used in IBD are aminosalicylates, antibiotics, immunomodulators and steroids (Carter *et al.,* 2004). The issues of these conventional drugs include side effects, complications and chance of recurrence of disease (Fernando *et al.* 2009). Use of steroids in IBD can lead to serious long life effects including high blood pressure, weight gain and susceptibility with infection. Immunosuppressant drugs may lead to birth defects and aminosalicylates may cause kidney problems. In line with above findings, the search has been extended for the use of acupuncture and herbal medicines which offer better protection and decrease the incidence of reoccurrence of IBD. However, acupuncture and use of massage have certain curative effects and fewer side effects compared to pharmacotherapy. Acupuncture therapy has been found effective in relieving the abdominal pain in IBD in humans (Xing *et al.*,2004). It is a non-drug approach to treat IBD which is safe and effective if used by a trained and skillful person. EA is simply driven method and can be easily controlled and can easily be used clinically. EA has been considered as very interesting technique and has been validated after its use in different study models (Vickers and Linde, 2014). EA is becoming widely used to treat different

gastro-intestinal disorders because of convenience use, satisfactory affects and very few side effects (Pie *et al.,* 2009) Analgesic affects by using EA can be obtained by activation of descending inhibitory mechanism involving serotonergic, opoidergic and adrenergic pathways in both peripheral and central nervous system (Han, 2003). Use of EA has been resulted to alleviate the hypersensitivity of intestine and colon (Wan *et al.,* 2017). Meta-analysis study showed a significant effect of acupuncture for treatment of IBS symptoms (Chao *et al.,* 2014). Severity and frequency of IBD symptoms have been lowered by acupuncture and the quality of life of IBS patients has been improved (Sun *et al.,* 2011). Repeated application of EA has effectively alleviate the chronic VH by decreasing Interleukins and can be used as an alternative therapeutic for the treatment of chronic VH (Wan *et al.,* 2017). The results of all these studies suggest acupuncture as a capable complementary cure of IBD hypersensitivity and pain. It is the need of hour to develop credible information to authenticate the effectiveness of acupuncture in Visceral Hypersensitivity and to recognize the basic mechanism. According to the World Health Organization report, herbal medicines are of great value for the health of communities.

The use of natural plants for treatment of different diseases has been seriously be considered (Koehn *et al.,* 2005). Indian plants and their extracts have been an important source for treating many diseases including IBD and several plants have shown an encouraging result for the treatment of IBD. Etienne *et al.* (1989) tested the effect of Gingko biloba on IBD and Chakurski *et al. (*1981) observed the effect of *Hypericum perforatum, Melissa officinalis, Calendula officinalis, Foeniculum vulgar in IBD.* Similarly, the extracts of some other medicinal plants were tested for the treatment of IBD by Kathleenand Julie (2003).The seeds of *N. sativa* and *E. caryophyllata* have been commonly used as herbal medicine throughout the world for the treatment of various diseases. Modern phyto-therapy has provided evidences about the beneficial effects of these plants for the treatment of IBS (Rahimi and Abdollahi, 2012). *Nigella sativa* commonly known as Black seed is an annual plant that has been traditionally used for the treatment of many diseases. *Nigella sativa* seedhas both fixed oil (30%) and volatile oil (0.4%) (Ali *et al.,* 2003). The oil of these plants have shown anti-inflammatory effects in many inflammatory models in rats (Isik *et al.,* 2011). The oil of *N. sativa* has shown an immonumodulatory effect by increasing the level of T-cell and Natural killer cell mediated immune response (Salem *et al.,* 2005) In light of these findings, the effect of *N. sativa* and *E. caryophyllata* oil to ameliorate the visceral hypersensitivity in dogs have been determined.

Surgical interventions can be effective to treat chronic inflammatory bowel disease and improve the quality of life of patients. Studies have shown that anastomosis after surgical resection of the affected part of ileum and colon has longer delayed the reoccurrence of CD in humane patients (Norton *et al.,* 2013). Usually surgery is indicated in case of failure of medical treatment, intestinal perforation, obstruction and hemorrhage (Rieder *et al.,* 2013). Resection of perforated part of intestine or colon decrease the mortality rate in CD as compare to simple closure with suturing (Werbin *et al.,* 2003). In equine, the surgical resections of affected bowel have been found effective in two cases of IBD having focal enteritis (Kalck, 2009)

The present study in dogs will contribute to the existing scientific knowledge by establishing an IBD Model of dogs and to document the effects of different therapeutic protocols including use of electro-acupuncture, *N. sativa*, *E. caryophyllata* and surgical resection of ileum against visceral hypersensitivity.

MATERIALS AND METHODS

***Animal grouping and methodology*:** The study was carried out during year 2018-19. Thirty clinically healthy dogs about one year of age were selected for this trial. Out of 30 dogs, 15 dogs were male and 15 dogs were female. Five random groups viz. Saline group, TNBS group, TNBS+EA group, TNBS + *Nigella sativa* and TNBS + *Eugenia caryophyllata* with six dogs in each group (n=6) were made.

***Induction of ileitis by TNBS*:** I ml solution containing 30mg of TNBS in 30 % ethanol was injected into ileal wall of dogs of TNBS group, TNBS+ EA group, TNBS + *Nigella sativa* group and TNBS + *Eugenia caryophyllata* group. Solution was injected at four points of ileum leaving 5cm of ileum from ileoceacal junction using 30 gauge needle. In saline group, an equal quantity of saline was injected instead of TNBS solution. Animals were Weight at day 0, 3, 6, 9, 12, 15, 18 and 21 following surgery.

***EA stimulation*:** Dogs of TNBS+EA group were slightly restrained and their hind legs were exposed bilaterally for EA treatment. Four acupuncture needles (0.4 mm in diameter and 75 mm in length) were inserted into bilateral Zu San Li (ST36) acupoint located 3 cm (1 cm ¼ width of the last rib) distal to the lateral head of the fibula. These needles were connected with EA apparatus (Multifunctional electrical pulse generator (G6805-3), Hua Yi Medical Instrument Factory, Shanghai, China). Dogs in the EA group were undergone the electro-acupuncture for 30 min (with frequencies 10 Hz and intensity of 2 to 3 mA) on day 6, 9, 12, 15,18 and 21 after TNBS treatment.

***Oral administration of Nigella sativa oil and Eugenia caryophyllata oil*:** The dogs in TNBS+ *N. sativa* and TNBS+ *E. caryophyllata* group were orally administered with *N. sativa* oil and *E. caryophyllata* oil @2.5 ml /kg on days 6, 9, 12, 15,18 and 21.

Visceral Hypersensitivity Reflection by Colorectal Distension (CRD) Testing: Visceral Hypersensitivity in dogs of all groups was reflected by Pain Response Score (PRS). Pain response score was assessed by monitoring Behavioral

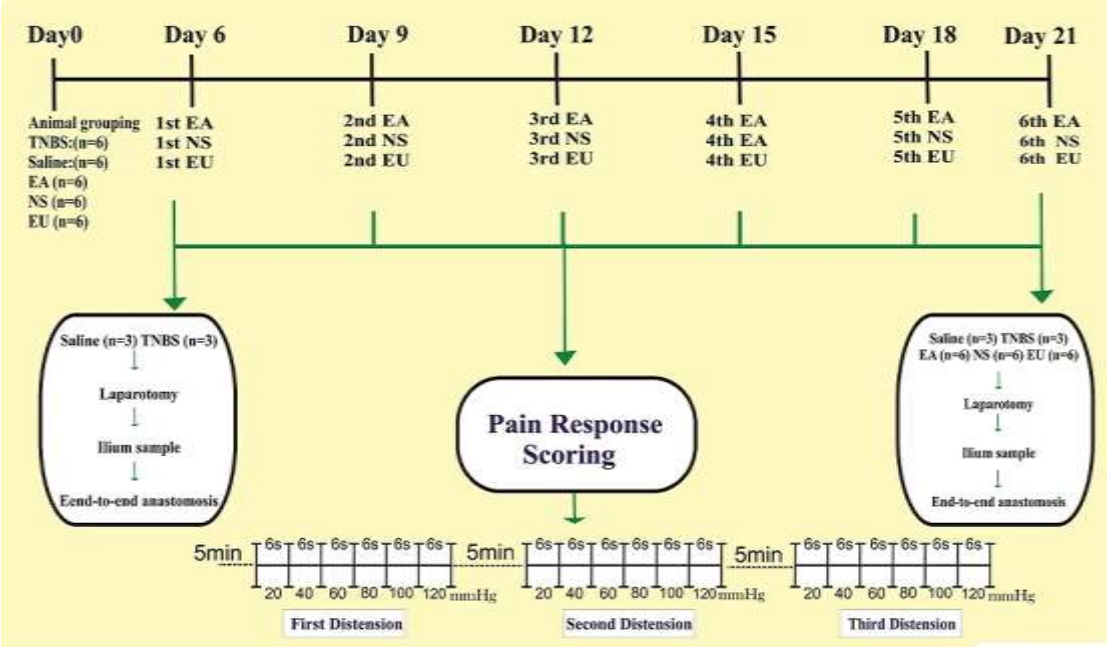

Figure 1. The model experiment design.

Pain Responses to Colorectal Distension (CRD) at day 6, 9, 12, 15, 18 and 21 after surgery and treatments in all groups. A scale of 0, 1, 2, 3 and 4 was used to quantify the Pain response after insertion of colorectal distension device for this study. After lubrication, the polyethylene balloon part of device was placed into the distal colon about 10 cm from the anus of dog. After 5 minutes of acclimatization, the balloon of device was inflated with pump. Pressure on the balloon was measured by sphygmomanometer. Balloon pressure was continuously increased from 20 to 120 mmHg stage by stage and lasted for 6 seconds at each stage. Pain response scour (PRS) was measured at each stage. Two observers noted the Pain response score of dogs who were blinded to the experimental conditions. Three readings of pain response score were noted at each stage and average value of three readings was used.

***Plasma level of cytokine Interluken-6 (IL-6)*:** Blood samples were taken from each group of dogs on day 6, 9, 12, 15, 18 and 21 after colorectal distension test. Plasma level of IL-6 Concentrations was measured using commercially available ELISA kit (mlbio canine interleukin 6, China) following the manufacturer's instructions

***Sampling of ileal tissues*:** Three dogs from Saline group (n=3) and three dogs from TNBS group (n=3) were passed through Laparotomy procedure to expose the ileum at day 6. 4-cm piece of ileum was resected leaving 5 cm from the junction of ileum and caecum. While remaining 3 dogs of Saline group and TNBS group and all dogs of EA, NS and EU groups were passed through the same surgical procedure on day 21 to get the ileum samples. These resected pieces of ileum were washed with PBS and longitudinal cuts were made. The macroscopic changes on mucosa were observed. 1.5×1.5 cm sized tissue sample were stored in 10% formalin. End-to-end anastomosis of ileum was performed immediately after sampling.

***Gross and Histological observations*:** Macroscopic lesions were scored separately and were assessed by ileal strictures, adhesions with neighboring tissues, Ulcers on mucosal membrane and thickness of ileal wall (Tahir *et al.,* 2015). For microscopic studies, Samples were processed by routine techniques. Three series of 5 µm-thickness tissue sections were made and staining of slides was made by using Heamatoxylin-Eosin (HE) stain (Tahir *et al.,* 2015). Microscopic scoring was done by two pathologists separately. The scoring criteria was fixed on the basis of tissue edema, infiltration of inflammatory cells, ulcerations and villus fusion

***Effect of surgical resection of ileum on abolishment of Visceral Hypersensitivity*:** A total of six dogs, three Dogs from Saline group (n=3) and three dogs (n=3) from TNBS group of previous experiment were further tested to evaluate the effect of surgical resection of treated part of ileum (with saline or TNBS) on Visceral Hypersensitivity. Laparotomy and end-to-end anastomosis of ileum was already performed on these dogs on day 6 of previous experiment to collect treated part of ileum for Grass and histological examinations. Visceral Hypersensitivity of dogs from both groups was reflected after Colorectal Distension Test on day 6, 9 and 12 after surgery by measuring pain response score (PRS).

***Statistical Analysis*:** For each experiment, Mean ± SD was used for expression of experimental data. Data were analyzed by using SPSS software. One way ANOVA (Parametric or

Non parametric) 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. Repeated Measured Design was performed for comparing the treatment groups with control. Results were considered significant when the *p*-values were less than 0.05.

RESULTS

After induction of ileitis with TNBS/Ethanol in TNBS, EA, NS, EU groups, dogs showed the Effect of TNBS induced ileitis on body weight sign of vomiting, diarrhea, loss of appetite, increase the frequency of feces. Body weights of dogs of all four groups were decreased significantly ($p<0.05$) as compare to saline group on day 6 of induced ileitis. On day 21, the body weights of all four groups were not significantly different from the saline group.

***Macroscopi cand microscopic changesofthe TNBS-treatedileum*:** Onday 6, macroscopic lesion score were found increased ($p<0.05$) in TNBS group (6.00±0.52), than in saline group (1.89±0.40). In the TNBS group, mild congestion of ilealmucosa and thickening of wall of ileum was observed. The adjacent organs did not show any apparent lesion. However, no difference inmacroscopic lesionscore between TNBS and Saline treated group was observed at day 21.

Microscopically at day 6, microscopic lesion scores of TNBS group was significantly higher along with mucosal and submucosal ulceration. While on day 21, the ileal wall showed moderate infiltration of inflammatory cells in the sub

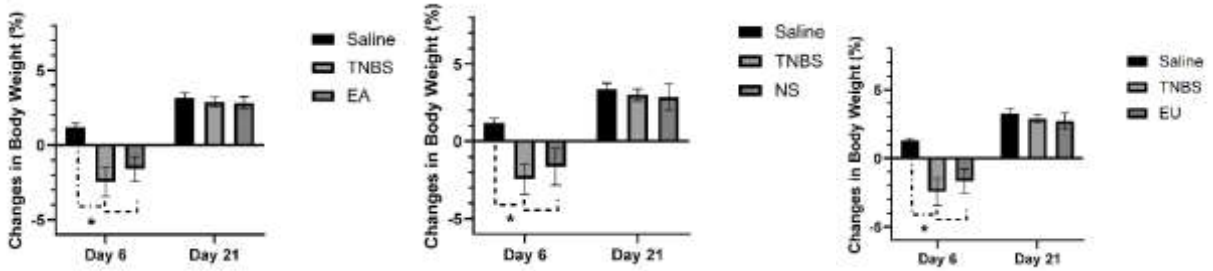

Figure 2. Effects of ileitis, EA treatments, NS Treatments and EU Treatments on body weight of Dogs. Changes in body weight were observed on day 6 and 21 of treatments.

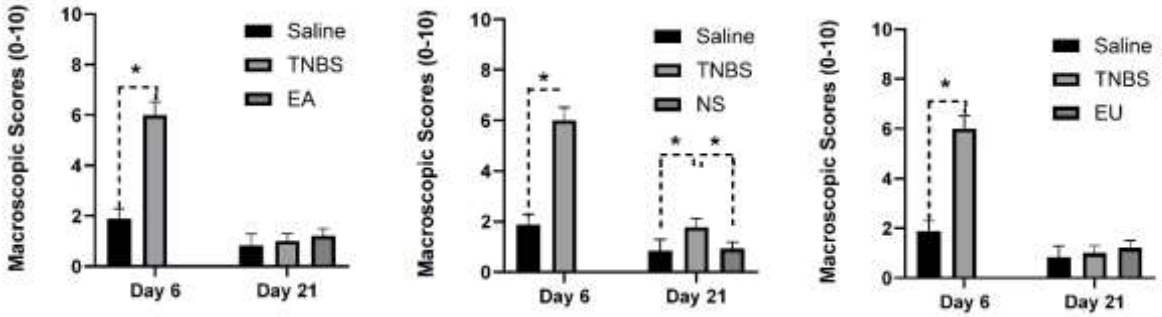

Figure 3. Effects of ileitis, EA treatments, NS treatments and EU treatments on macroscopic lesion score of dogs. Changes in macroscopic lesions scores at day 6 and 21 of treatments.

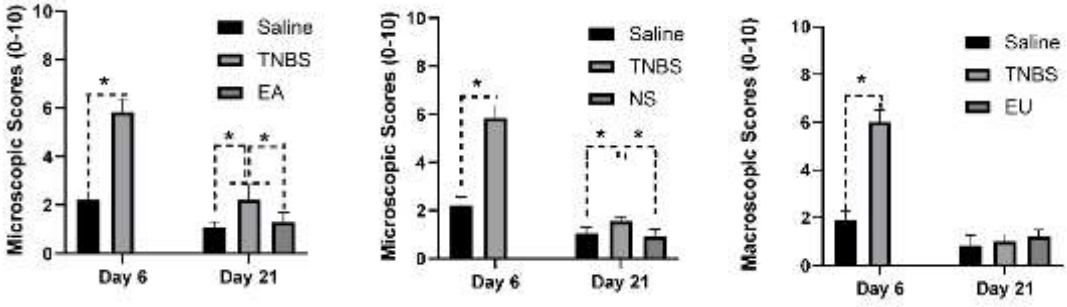

Figure 4. Effects of ileitis, EA treatments, NS treatments and EU treatments on microscopic lesion score of dogs. Changes in microscopic lesions scores at day 6 and 21 of treatments

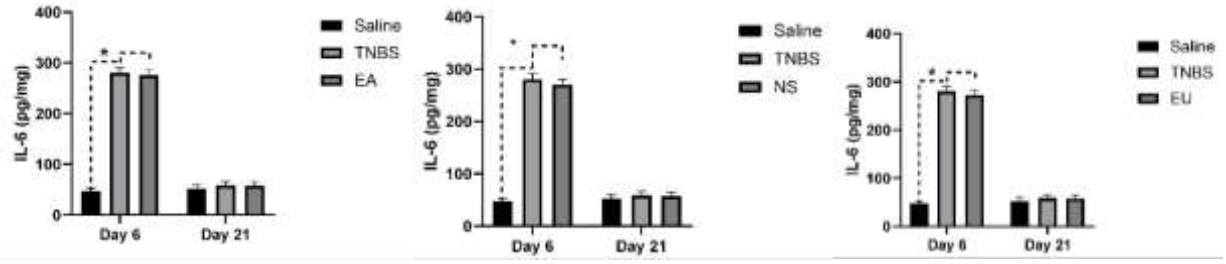

Figure 5. Effects of ileitis, EA treatments, NS treatments and EU treatments on plasma concentration of IL-6 of dogs. Changes in the mean values of plasma concentration of IL-6 at day 6 and 21 of treatments.

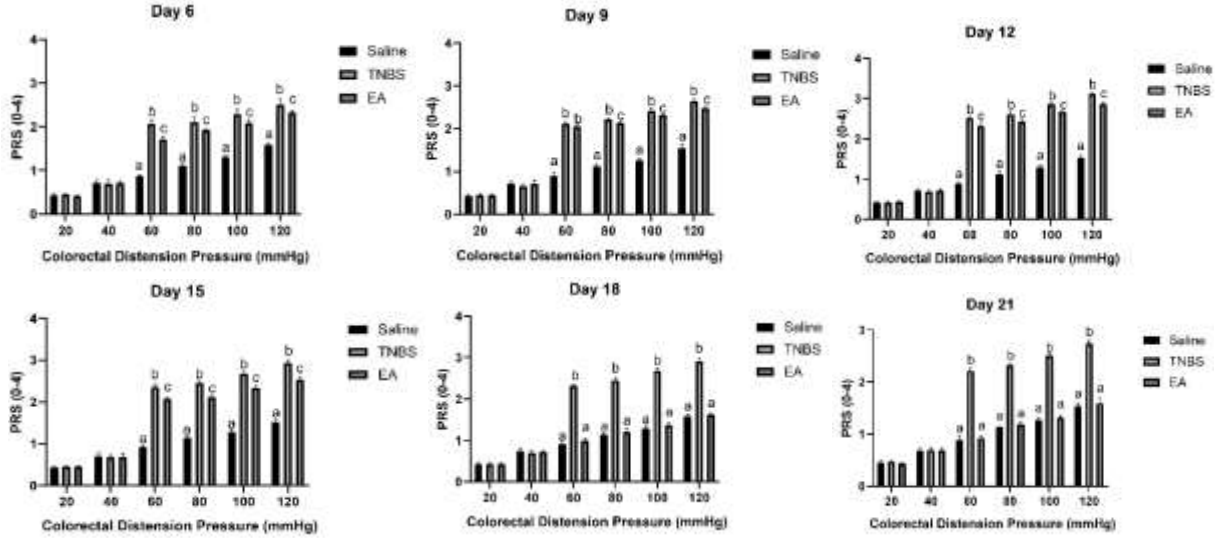

Figure 6. Effects of repeated Electro-Acupuncture treatments on Pain Response Score (PRS) at day 6, 9, 12, 15, 18, and 21.

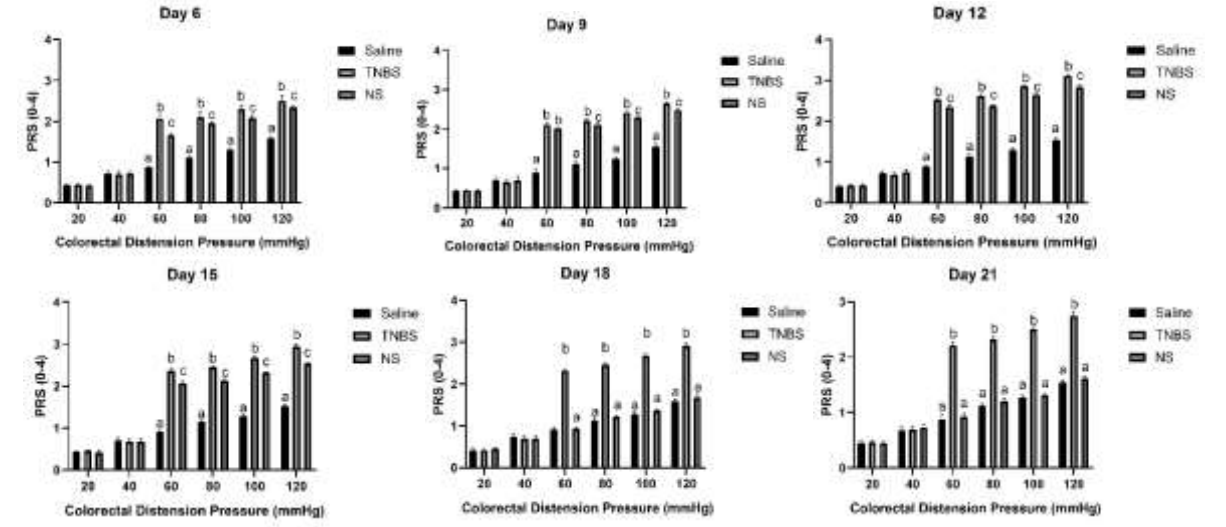

Figure 7. Effects of oral administration of *Nigella sativa* treatments on Pain Response Score (PRS) at day 6, 9, 12, 15, 18, and 21.

mucosa. No significant difference in microscopic lesion score was observed among all the groups at day 21.

***Effect of ileitis on Plasma concentration of IL-6*:** Plasma concentration of IL-6 was found significantly higher (280.38±10.28 pg/ml) in TNBS treated group as compare with saline group (47.50±5.28 pg/ml) on day 6 of induction

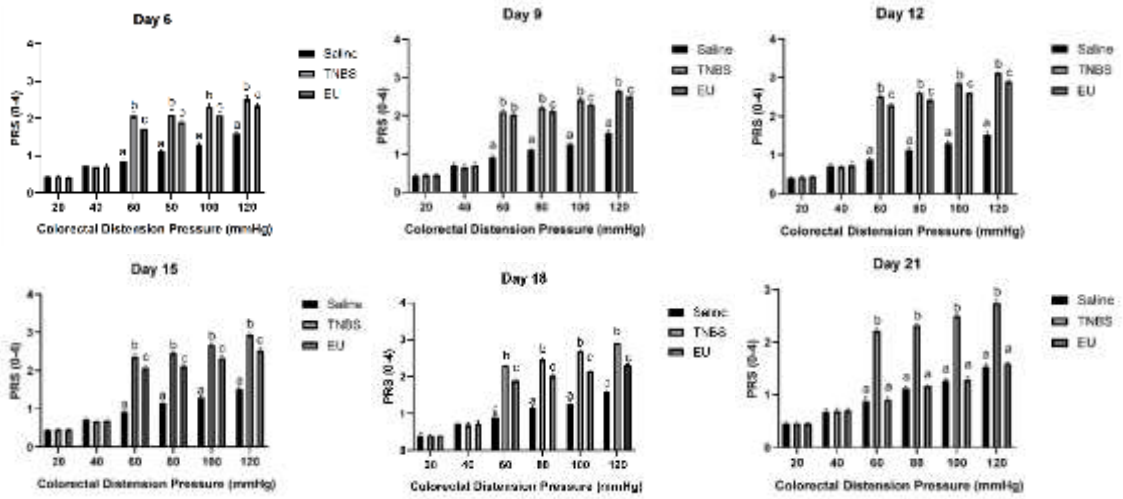

Figure 8. Effects of oral administration of *Eugenia caryophyllata* oil treatments on Pain Response Score (PRS) at day 6, 9, 12, 15, 18, and 21.

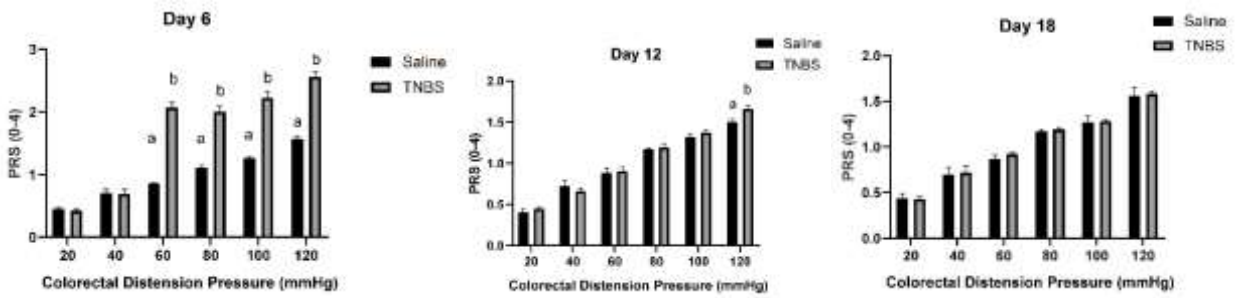

Figure 9. Effects of Surgical treatment on Pain Response Score (PRS) at day 6, 12 and 18.

of ileitis. On day 21, no significant difference was found between the mean values of plasma concentration among all four groups.

Effect of repeated use of Electro-acupuncture (EA), Nigella sativa (NS) and Eugenia (EU) oil on pain response score (PRS) to colorectal distension: Pain response score was measured with graded Colorectal Distension Deviceat20,40,60,80, 100 and 120 mmHg). The dogs showed signs such as agitation, change of position, backing of head to the abdomen and vocalization when balloon distension pressure was applied. Increased PRS was observed in TNBS-treated dogs (p<0.05) than saline- treated dogsatday6, 9, 12,15,18and21exceptfor thatwith20 mmHg and 40 mmHg. In EA treated group, PRS was higher at 60-120 mmHg pressure on day 6-9. It was higher at 80-120 mmHg at day 12-15 and was similar with the control group from day 18-21 at pressure from 60-120 mm Hg. In NS treated group, the PRS was higher at 60-120 mmHg pressure on day 6-12. It was higher at 80-120 mmHg at day 15 and was similar with control group from day 18-21 at the pressure of 60-120 mmHg. In EU treated group, the PRS was higher at 60-120 mmHg pressure on day 6-15. It was higher at 80-120 mmHg at day 18 and was similar with control group on day 21 at pressure of 60-120 mmHg.

***Effect of surgical treatment on Visceral Hypersensitivity*:** Visceral Hypersensitivity of dogs from both groups viz. saline group and TNBS treated group was reflected after Colorectal Distension Test on day 6, 9 and 12 after surgical resection of treated part of ileum by measuring pain response score (PRS). On day 6, PRS was higher at 60-120mmHg pressure in TNBS treated group as compared to saline group, it was significantly higher on day 12 at 120 mm Hg. On day 18, there was no significant difference in PRS between Saline and TNBS treated groups.

DISCUSSION

Visceral hypersensitivity (VH) models have been developed in various animals to study the various parameters of visceral hypersensitivity. Bradesi *et al. (*2005) established VH in rats by keeping water avoid stress. (Al–Chaer *et al.*, 2000) observed VH in neonatal rats by giving colorectal distension. Shah *et al. (*2016) used the injection of TNBS/ethanol into lumen of ileum of rats and established ileitis with VH. Janyaro

*et al. (*2016) and Tahir *et al*. (2015) developed VH Model in goats by injecting TNBS solution in the wall of goat ileum. Present study has been conducted on dogs to produced ileitis along with VH to observe the effect of different treatments including the application of EA, oral administration of *N. sativa* and Eugenia oil and surgical resection of affected part of ileum. 1ml of 30 mg TNBS in 30% ethanol was injected into the ileal wall of all Dogs except the control group in which 1 ml of normal saline was injected. Dogs developed in appetence, vomiting, diarrhea, weight loss, increase levels of IL-6, and microscopically the ileum developed infiltration of inflammatory cells, thickening of the affected wall of ileum, necrosis and adhesion of ileum and ulcer formation at day 6. These findings are consistent with the findings of Janyaro *et al. (*2016) and Tahir *et al. (*2015). In our study, VH was observed from day 6 of TNBS induced ilietis and on day 12 it get maximize in term of Pain Response Scoring and it remained at the same level to day 21. These findings are similar with studies conducted by Tahir *et al. (*2015), Shah *et al. (*2016) and Wan *et al.* (2017).

There are certain reports about the ameliorating effect of EA in VH (Cui *et al.,* 2005; Liu *et al.,* 2009). Cheng *et al. (*2012) established the fact that 60 Hz frequency of EA can induced the optimal analgesia effect in goats. Acupoint at Zusanli has been used affectively for analgesia and gastrointestinal disorders (Han, 2003; Xu *et al.,* 2009). Liu *et al. (*2009) successfully suppressed the chronic VH by repeated application of EA at bilateral Zusanli (Wang *et al.,* 2002) studied the comparative analgesic effects of Electro-Acupuncture with use of different intervals. He observed that at 2-days interval best cumulative analgesic effect was attained. In our study, 10 Hz Electro-Acupuncture was applied with the 3-days interval at bilateral Housanli acupoints. It significantly reduced the ileitis-induced pain response score in dogs. Maximum effect for relieving the VH was attained after five treatments (day 6, 9,12,15,18 and 21) on day 21. These results are consistent with outcomes of other studies (Cui *et al.,* 2005; Tahir *et al.,* 2015; Shah *et al.,* 2016). Chu *et al.* (2012) and Zhuang *et al. (*2016) found an increase level of Pro-inflammatory cytokine (IL-6) as a result of peripheral and central inflammations due to mechanical and thermal stimuli. Liu *et al. (*2012) confirmed that chronic pain provoked the up regulated expression of pro-inflammatory cytokine (IL-6). Arruda *et al. (*2000) and Dominguez *et al. (*2010) also established the fact that chronic pain provoked the increased concentration of IL-6. In present study, an increased level of IL-6 has been found due to TNBS induced ileitis that may be associated with the development of Visceral Hypersensitivity.

Among many auspicious medicinal plants being studied by researchers, *Nigella sativa* has been documented as one of the most auspicious medicinal plants after many studies by different researchers. Thymoquinone (TQ) which is a volatile oil extracted from black of seed of NS has been used by workers in various inflammation- based animal models including experimental encephalomyelitis, edema, peritonitis, and arthritis and has been proved affective as antioxidant and anti-inflammatory agent (Kalus *et al.,* 2003; Salem, 2005). Tekeoglu *et al.* (2007) reported the anti-inflammatory effect of TQ on induced arthritis model in rats and found a decreased levels of circulating pro-inflammatory cytokines and amelioration of induced arthritis. El-Dakhakhny *et al. (*2000) found the amelioration of ethanol-induced ulcers in 53.56 % of rats by use of NS oil compared to the control. Similarly, Mahgoub established same effect of TQ against acetic acid-induced colitis in rats (Mahgoub, 2003). Isik *et al. (*2011) studied the effect of NS Oil for treatment of TNBS induced colitis in rats and established the fact that NS oil decreased the pro-inflammatory cytokine (IL-6). In the present study the NS Oil was used to evaluate its effect in regulating the inflammatory response in experimentally induced ileitis model of dogs with Visceral Hypersensitivity. An oral administration of NS oil prevented and reduced the TNBS induced ileitis in dogs. Our data indicates the capability of NS oil supplementation to reduce the TNBS induced ileitis by reducing the inflammatory activity by reducing the macroscopic and microscopic score as compare to control (Saline group).

A representative outcome of IBD is too much production of pro-inflammatory mediators such as interleukin-6 (Ravindran *et al.,*2010). These signaling molecules are responsible for development of the pathogenesis of IBD by activation of various phospholipases, sphingomyelinases, and other lipid-modifying enzymes (Burits and Bucar, 2000; El-Saleh *et al.,*2004). Intracolonic injection of TNBS in mice model (Kaseb *et al.,* 2007; Banerjee *et al.,* 2010) resulted into diffuse colonic inflammation, leukocyte infiltration, edema, and ulceration and increased in pro-inflammatory cytokines (Yi *et al.,* 2008). Bodaghi *et al.* (2019) reported that oral use of NS powder significantly decreased inflammatory factors in the IBD patients. In the present study, an increased level of IL-6 was observed in response to TNBS induced ileitis and was significantly decreased after oral administration of NS oil in dogs.

Eugenia caryophyllata is commonly known as "clove" is cultivated throughout the world. The seed and oil of *E. caryophallata* has been used for the treatment of various diseases. Traditionally it has been used for dental care, analgesic and antiseptic agent (Öztürk and Özbek, 2005). Taher *et al.* (2015) studied the anti-inflammatory effect of Clove oil and established the anti-inflammatory and anti-nociceptive activity of clove oil in mice. Current Phyto-therapy has provided information about the beneficial effects of this plant for the treatment of various diseases including Inflammatory Bowel Disease (Rahimi and Abdollahi, 2012). Bachiega *et al*. (2012) used the essential oil and extract of clove (euginol) to analyze the immunomodulatory and anti-inflammatory effect on cytokines production (IL-6 and IL-

10). Form their finding it was observed that both have a significant effect in inhibiting the production of cytokines by murine macrophages.

The results of present study extend our knowledge about the use of *E. caryophyllata* oil (clove oil) in ameliorating the TNBS induced ileitis with visceral hypersensitivity in dog. *Eugenia* oil was orally administered @ 2.5 ml per kg at day 6, 9, 12, 15,18 and21 after induction of ileitis by intramural injection of 1ml of 30 mg Solution of TNBS in 30% ethanol. There was a significant reduction in microscopic and macroscopic lesions score compare with TNBS group in which no oral administration of Eugenia oil was given. Similarly, Plasma level of Interluken-6 was significantly decreased in Eugenia group. All these findings show that *E. caryophyllata* oil can ameliorate the TNBS induced ileitis in dogs. Similarly, there was significant reduction in Pain response score (PRS) in dogs treated with Eugenia oil as compare to TNBS treated from day 18-21 after the treatment of eugenia oil. These findings are consistent with study of Park *et al. (*2011) about the analgesic effect of oral administration of eugenol. An oral and Intra-peritonial administration of *Eugenol* was found effective in reducing the number of acetic acid-induced abdominal constrictions. Both routes of administration yielded with similar effects. The good results may be due to pharmacokinetics (e.g., good bioavailability of eugenol by oral route (Guénette *et al.*, 2007) In our study, the *Eugenia* oil was given orally in dogs and good efficacy to ameliorate the VH was observed.

Studies suggest administration of TNBS GIT can provoke release of endogenous inflammatory mediators like cytokines (e.g., TNF-a, IL-1b, IL-6) from resident macrophages and mast cells in the abdominal cavity, which stimulate nociceptive neurons (Collier *et al.*, 1968; Park *et al.*, 2011). Oral administration of eugenol has an antinociceptive effect against acetic acid- induced nociception (Vikman *et al.*,2005: Batista *et al.*, 2010). The results of our study also demonstrate the development of nociception and visceral hypersensitivity resulting from TNBS induced ileitis and increased level of cytokine (IL-6). *Eugenia* oil oral administration effectively ameliorate the VH by decreasing the level of IL-6, which suggests Eugenia oil can inhibit response triggered by cytokines.

***Conclusions*:** Repeated application of EA at bilateral Zu San Li (ST36) acupoint and oral administration of *Nigella sativa* and *Eugenia caryophallata* oil attenuated the TNBS/Ethanol-induced ileitis along with visceral hypersensitivity by reducing plasma IL-6 level, microscopic, Macroscopic ileal tissue damage score and pain response score. Surgical resection of affected part of ileum also alleviated the visceral hypersensitivity by significantly decreasing the pain response scoring in dogs. So repeated application of EA, oral administration of *N, sativa* and *E. caryophyllata* oil and surgical resection of affected part of ileum could be potentially used to treat the chronic VH in dogs.

REFERENCES

Al–Chaer, E.D., M. Kawasaki and P.J. Pasricha. 2000. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. Gastroenterol. 119:1276-1285.

Ali, B.H. and G.Blunden. 2003. Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res.: An Int. J. Dev. Pharmacol. Toxicol. Eval. Nat. Prod. Deriv. 17:299-305.

Arruda, J.L., S. Sweitzer, M.D. Rutkowski and J.A. DeLeo. 2000. Intrathecal anti-IL-6 antibody and IgG attenuates peripheral nerve injury-induced mechanical allodynia in the rat: possible immune modulation in neuropathic pain. Brain Res. 879:216-225.

Bachiega, T.F., J.P.B. de Sousa,J.K. Bastos and J.M. Sforcin. 2012. Clove and eugenol in noncytotoxic concentrations exert immunomodulatory/anti inflammatory action on cytokine production by murine macrophages. J. Pharm. Pharmacol. 64:610-616.

Banerjee, S., S. Padhye, A. Azmi, Z. Wang, P.A. Philip, O. Kucuk, F.H. Sarkar and R.M. Mohammad. 2010. Review on molecular and therapeutic potential of thymoquinone in cancer. Nutr.Cancer 62:938-946.

Batista, P. A., M.F. de Paula Werner, E.C. Oliveira, L. Burgos, P. Pereira, L.F. da Silva Brum, G.M. Story and A.R. Santos. 2010. The antinociceptive effect of (-)-linalool in models of chronic inflammatory and neuropathic hypersensitivity in mice. J. Pain. 11:1222-1229.

Bradesi, S., I. Schwetz, H.S. Ennes, C.M. Lamy, G. Ohning, M. Fanselow, C. Pothoulakis, J.A. McRoberts and E.A. Mayer. 2005. Repeated exposure to water avoidance stress in rats: a new model for sustained visceral hyperalgesia. Am. J. Physiol. Gastrointest. Liver Physiol. 289:42-53.

Burits, M. and F. Bucar. 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14:323-328.

Carter, M.J., A.J. Lobo and S.P. Travis. 2004. Guidelines for the management of inflammatory bowel disease in adults. Gut. 53:1-16.

Chakurski, I., M. Matev andA. Koichev. 1981. Treatment of chronic colitis with an herbal combination of *Taraxacum officinale*, *Hipericum perforatum*, *Melissa officinalis*, *Calendula officinalis* and *Foeniculum vulgare*. Vutreshni Bolesti. 20:51-54

Chao, G.Q. and S. Zhang. 2014. Effectiveness of acupuncture to treat irritable bowel syndrome: a meta-analysis. World J. Gastroenterol. 20:1871-7.

Cheng, L.-L., M.-X. Ding, C. Xiong, M.-Y. Zhou, Z.-Y. Qiu andQ. Wang. 2012. Effects of electroacupuncture of

different frequencies on the release profile of endogenous opioid peptides in the central nerve system of goats. Evid. Based Complementary Altern. Med. https://doi.org/10.1155/2012/476457

Chu, H., J. Sun, H. Xu, Z. Niu and M. Xu. 2012. Effect of periaqueductal gray melanocortin 4 receptor in pain facilitation and glial activation in rat model of chronic constriction injury. Neurol.Res.34:871-888.

Collier, H., L. Dinneen, C.A. Johnson and C. Schneider. 1968. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br.J.Pharmacol.Chemother.32:295-310.

Cui, K., W. Li, X. Gao, K. Chung, J. Chung and G. Wu. 2005. Electro-acupuncture relieves chronic visceral hyperalgesia in rats. Neurosci. Lett. 376:20-23.

Dominguez, E., A. Mauborgne, J. Mallet, M. Desclaux and M. Pohl. 2010. SOCS3-mediated blockade of JAK/STAT3 signaling pathway reveals its major contribution to spinal cord neuroinflammation and mechanical allodynia after peripheral nerve injury. J. Neurosci. 30:5754-5766.

El-Dakhakhny, M., M. Barakat, M.A. El-Halim and S. Aly. 2000. Effects of Nigella sativa oil on gastric secretion and ethanol induced ulcer in rats. J.Ethnopharmacol. 72:299-304.

El-Saleh, S.C., O.A. Al-Sagair and M.I. Al-Khalaf. 2004. Thymoquinone and *Nigella sativa* oil protection against methionine-induced hyperhomocysteinemia in rats. Int. J. Cardiol. 93:19-23.

Etienne, A., F. Thonier and P. Braquet. 1989. Protective effect of the PAF-antagonist BN 52021 on several models of gastro-intestinal mucosal damage in rats. Int. J. Tissue React. 11:59- 64.

Fernando, G. and P.G. Javier. 2009. Anemia and inflammatory bowel diseases. World J. Gastroenterol. 15:4659-65.

Guénette, S.A., A. Ross, J.-F.Marier, F. Beaudry and P. Vachon. 2007. Pharmacokinetics of eugenol and its effects on thermal hypersensitivity in rats. Eur.JPharmacol. 562:60-67.

Han, J.-S.2003. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci. 26:17-22.

Isik, F., T.T. Akbay, A. Yarat, Z. Genc, R. Pisiriciler, E. Caliskan-Ak, S. Cetinel, A. Altıntas and G. Sener. 2011. Protective effects of black cumin (*Nigellasativa*) oil on TNBS-induced experimental colitis in rats. Digest. Dis.Sci. 56:721-730.

Janyaro, H., J. Wan, A.H. Tahir, M.K. Shah, X.-J. Li and M.-X. Ding. 2016. Visceral pain triggered by traction on the ileocecal ligament with ileitis. J. Pain Res. 9:745.

Kalck, K.A. 2009. Inflammatory bowel disease in horses. Vet. Clin.: Equine Pract. 25:303-315.

Kalus, U., A. Pruss, J. Bystron, M. Jurecka, A. Smekalova, J.J. Lichius and H. Kiesewetter. 2003. Effect of *Nigella sativa* (black seed) on subjective feeling in patients with allergic diseases. Phytother. Res. Int. J. Dev. Pharmacol. Toxicol. Eval. Nat. Prod. Derivat. 17:1209-1214.

Kaseb, A.O., K. Chinnakannu, D. Chen, A. Sivanandam, S. Tejwani, M. Menon, Q.P.Dou and G.P.-V.Reddy. 2007. Androgen receptor–and E2F-1–targeted thymoquinone therapy for hormone-refractory prostate cancer. Cancer Res. 67:7782-7788.

Kathleen, H. and S. Julie. 2003. Inflammatory Bowel Disease Part I: Ulcerative Colitis – Pathophysiology and Conventional and Alternative Treatment Options. Alt. Med. Rev. 8:247-83.

Koehn, F.E. and G.T. Carter. 2005. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov. 4:206-20.

Liu, H.-R., X.-M.Wang, E.-H. Zhou, Y. Shi, N. Li, L.-S.Yuan and H.-G.Wu. 2009. Acupuncture at both ST25 and ST37 improves the pain threshold of chronic visceral hypersensitivity rats. Neurochem.Res. 34:1914-1918.

Mahgoub, A.A. 2003. Thymoquinone protects against experimental colitis in rats. Toxicol. lett. 143:133-143.

Norton, C., L.B. Dibley and P. Bassett. 2013. Faecal incontinence in inflammatory bowel disease: associations and effect on quality of life. J. Crohn's Colitis. 7:e302-11.

Bodaghi, M.N., Z. Darabi, S. Agah and A. Hekmatdoost. 2019. The effects of *Nigella sativa* on quality of life, disease activity index, and some of inflammatory and oxidative stress factors in patients with ulcerative colitis. Phytother. Res. 33:1027-1032.

Öztürk, A. and H. Özbek. 2005. The anti-inflammatory activity of *Eugenia caryophyllata* essential oil: an animal model of anti-inflammatory activity. Eur. J. Gen. Med. 2:159-163.

Park, S.-H., Y.-B.Sim, J.-K.Lee, S.-M.Kim, Y.-J.Kang, J.-S.Jung and H.-W.Suh. 2011. The analgesic effects and mechanisms of orally administered eugenol. Arch.Pharmacal Res. 34:501-507.

Pie, H. and H.R. Weng. 2009. Glutamate transporters prevent excessive activation of NMDA receptors and extrasynaptic glutamate spillover in the spinal dorsal horn. J. Neurophysiol. 101:2041-2051.

Rahimi, R. and M. Abdollahi. 2012. Herbal medicines for the management of irritable bowel syndrome: a comprehensive review. World J.Gastroenterol. 18:589.

Ravindran, J., H.B.Nair, B. Sung, S. Prasad.R.R. Tekmal and B.B. Aggarwal. 2010. RETRACTED: Thymoquinone poly (lactide-co-glycolide) nanoparticles exhibit enhanced anti-proliferative, anti-inflammatory, and chemosensitization potential. pp.1640-1647.

Rieder, F., E.M. Zimmermann, F.H. Remzi and W.J. Sandborn. 2013. Crohn's disease complicated by strictures: a systematic review. Gut. 62:1072-84.

Salem, M.L. 2005. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int.Immunopharmacol. 5:1749-1770.

Shah, M.K., J. Wan, H. Janyaro, A.H. Tahir, L. Cui, M.-X.Ding. 2016. Visceral hypersensitivity is provoked by 2, 4, 6-trinitrobenzene sulfonic acid-induced ileitis in rats. Front. Pharmacol. 7:214.

Sun, J.H., X.L. Wu and C. Xia. 2011. Clinical evaluation of Soothing Gan and invigorating Pi acupuncture treatment on diarrhea-predominant irritable bowel syndrome. Chin. J. Integr. Med. 10:780-5

Taher, Y.A., A.M. Samud, F.E. El-Taher,G. ben-Hussin,J.S. Elmezogi, B.F. Al-Mehdawi and H.A. Salem. 2015. Experimental evaluation of anti-inflammatory, antinociceptive and antipyretic activities of clove oil in mice. Libyan J. Med.10:28685. http://dx.doi.org/10.3402/ljm.v10.28685.

Tahir, A.H., Wan, J., Shah, M. K., Janyaro, H., Li, X.-J., and Ding, M.-X. (2015). A novel model for studying ileitis-induced visceral hypersensitivity in goats. Acta Vet. Scand. 58:72-??.

Tekeoglu, I., A. Dogan, L. Ediz, M. Budancamanak and A. Demirel. 2007. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. Phytother. Res. Int. J. Dev. Pharmacol. Toxicol. Eval. Nat. Prod. Derivat.21:895-897.

Vickers, A.J. and K. Linde. 2014. Acupuncture for chronic pain. J. Am. Med. Assoc. 311:955-956.

Vikman, K., P. Siddall and A. Duggan. 2005. Increased responsiveness of rat dorsal horn neurons in vivo following prolonged intrathecal exposure to interferon-γ. Neurosci. 135:969-977.

Wan, J., Y. Ding, A.H. Tahir, M.K. Shah, H. Janyaro, X. Li, J. Zhong, V. Vodyanoy and M. Ding. 2017. Electroacupuncture attenuates visceral hypersensitivity by inhibiting JAK2/STAT3 signaling pathway in the descending pain modulation system. Front.Neurosci. 11:644-???.

Wang, H., Y. Wan, L. Yao and J. Han. 2002. Comparison of the therapeutic effects of electroacupuncture with different interval for treatment of chronic neuropathic pain in rats. Acupunc. Res. 27:112-118.

Werbin, N., R. Haddad, R. Greenberg, E. Karin and Y. Skornick. 2003. Free perforation in Crohn's disease. Isr. Med. Assoc. J. 5:175-7.

Xing, J., B. Larive, N. Mekhail and E. Soffer. 2004. Transcutaneous electrical acustimulation can reduce visceral perception in patients with the irritable bowel syndrome: a pilot study. Altern.Ther. Health Med. 10:38.

Xu, G.Y., J. Winston and J. Chen. 2009. Electroacupuncture attenuates visceral hyperalgesia and inhibits the enhanced excitability of colon specific sensory neurons in a rat model of irritable bowel syndrome. Neurogastroent. Motil. 21:1302-e1125.

Yi, T., S.-G. Cho, Z.Yi, X.Pang, M.Rodriguez, Y.Wang, G.Sethi, B.B.Aggarwal andM. Liu. 2008. Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. Mol.Cancer Ther. 7:1789-1796.

Zhuang, X., Y. Chen, X. Zhuang, T. Chen, T. Xing, W. Wang and X. Yang. 2016. Contribution of Pro-inflammatory cytokine signaling within Midbrain Periaqueductal gray to Pain sensitivity in Parkinson's Disease via gaBaergic Pathway. Front. Neurol. 7:104.

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