

Rabbits Colitis, Disease and Treatment: Suggestion for Research Study

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Various immunosuppressive drugs have been mainly used for treatment of inflammatory bowel disease (IBD); however, their side effects and toxicity remain a major clinical concern. As a result, there is an increasing interest in using herbal medicine as an alternative and adjunct treatment in addition to the conventional therapies. As a suitable rabbit colitis model is currently not available, we developed a rabbit colitis model by administration of dextran sodium sulphate (DSS). Extraction of Dill Oil (DO) and Fennel Oil (FO). White Himalayan rabbits were acclimatized for 7 days and then separated into five groups each of five rabbits. Group (1) (-ve) control, Group (2) DSS (+ve) control, Group (3) DSS+DO (1ml/day, 5%), Group (4) DSS+FO (1ml/day, 3.5%) and Group (5) DSS+DO+FO (0.5ml each per day). At the end of the experiment, rabbits will be sacrificed and the entire colon will be excised. Total glutathione will be determined in the colon homogenate as well as, total RNA will be isolated from the colon homogenate, quantitative real-time PCR (qRT-PCR) reactions will be performed. Quantification of gene expression, Rabbit-specific primers will be designed for the genes of interest. The housekeeping gene β -actin will be used as an internal control, and quantification of the transcripts will be performed by the $\Delta\Delta$ CT method. Data will be expressed as mean \pm standard error of means (SEM). Comparisons will be performed by one-way ANOVA. *Expected outcomes* Upon detection of new treatments will be the entrance to produce a potent therapeutic preparation can be manufactured locally and marketed after making stages rely followed.

Background and Significance

Inflammatory Bowel Disease and Treatment

Inflammatory bowel disease (IBD) is a chronic and degenerative inflammatory condition of the gastrointestinal (GI) tract that which manifested by ulcerative colitis (UC) or Crohn's disease (CD) [1]. IBD is associated with an increased danger of colon and rectal cancer resulting in a substantial liability to death [2]. Although the definite causes of IBD still remain unclear, it has been usually accepted that the extreme production of cytokines and mediators, such as interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α), has a vital role in the occurrence of IBD [3]. In recent times, agents have anti-TNF played a curious role in the clinical interference of IBD, However, not all patients respond to treatment, some become intolerant over time [4].

Nuclear factor (NF- κ B) is an essential mediator of inflammation and immunity which controls the transcription of cytokine and genes. Increased NF- κ B activation has been noticed in the mucosa of IBD and in murine IBD models [5]. An effective approach to avoid IBD in experimental models and preventing the production of pro-inflammatory cytokines in CD patients is the inhibition of NF- κ B with a particular oligonucleotide [6–8].

Various immunosuppressive drugs have been mainly used for the treatment of IBD; however, their side effects and toxicity remain a major clinical concern [9,10]. As a result, there is an increasing interest in using herbal medicines as an alternative and adjunct treatment in addition to the conventional therapies [11].

Dill "*Anethum graveolens* L." is a yearly herb which is local to Mediterranean countries and southeastern Europe that has been known in different systems of conventional medication for the treatment of different diseases in human [12]. The dill seeds have main constituents of essential oil that provide good antioxidant activities [13]. The flavonoids, essential oil, and phenolic compounds of this plant were screened by the phytochemical test and demonstrated the antibacterial, antioxidant antispasmodic, antiulcer and diuretic consequence [14].

Fennel "*Foeniculum vulgare*" is one of medicinal plants that belonged to Apiaceae family, Umbelliferae. The leaves, stalks and seeds of the plant are not poisonous. Many phytochemical research have been investigated the chemical component of the essential oil of fennel and have shown that the major components are phenylpropanoid and monoterpenoids [15]. As a result of the major pharmacological properties fennel oil, it has been used as an aromatic herb centuries in the Mediterranean area and also in folk medicine [16]. Fennel oil has many pharmacological activities including hepatoprotective, diuretic, anti-inflammatory, analgesic and antioxidant activities [17-19].

Diseases Associated with Rabbit Colitis

Escherichia coli shows to be associated with the rabbit disease. We now consider that the presence of large quantities of *E. coli* in the intestinal tract of rabbits is a requirement for the development of mucoid enteritis [20]. Moreover, colitis in rabbits may be related to *Clostridium difficile* or *Clostridium perfringens* iota toxins [21].

Additionally, Rabbits are an experimental model to *Clostridium difficile*-associated disease (CDAD), while *C. spiriforme* may be a more common cause of the disease. Peracute death and no other clinical signs of diseases are common characteristics of CDAD in rabbits [22]. While, European Commission [23] declared that *Mycobacterium avium* subsp. *Paratuberculosis* (Map) is an agent of Johne's disease which is a chronic inflammation of the intestine in livestock animals and rabbits. Furthermore, Rabbits can harbor the Enterohemorrhagic *Escherichia coli* (EHEC). The infectious dose in human is very low, which raise the risk of disease. Pathogenic *E. coli* that can cause diarrhea or hemorrhagic colitis in humans and acquire it by direct or indirect contact [24].

Acetic acid and dextran sodium sulfate were used as inducers of colitis in animal models in dogs and rabbits [25]. A chronic colitis has been induced in rabbits and have the same histological characters of human ulcerative colitis [26]. Chronic gut inflammation has an effect on the development of colon and rectal cancer and increased mortality [27]. Interleukin-1 decreased the inflammation before the colitis induction [28].

Aim of Work

In the present study, we will investigate the potential of Dill and Fennel oils in the treatment of rabbits with DDS-induced IBD, and examine their impact on the expression of NF- κ B gene and genes of pro-inflammatory cytokine IL-1B as well as apoptosis genes BAX and BCL-2 and antioxidant SOD2 gene.

Research Design and Methodologies

Induction of Colitis

Colitis was induced in rabbits by administering Dextran sulfate, sodium DSS (MW = 40 kDa; 50 mg/L) in the drinking water as described previously [29]. The experiment lasted for 10 days.

Experimental Design

White Himalayan rabbits (n=25) were acclimatized for 7 days and then separated into five groups each of five rabbits as follows:

Group (1) control, Group (2) DSS + control, Group (3) DSS + DO (1ml/day, 5 %), Group (4) DSS + FO (1ml/day, 3.5 %) and Group (5) DSS + DO + FO (0.5ml each per day) [21].

All groups had free access to food on days 1–29, and DSS was administered during the last week of the experiment in groups 2–5. All rabbits were killed the day after DSS treatment was discontinued – on day 30.

Colitis Evaluation

Animals will be monitored daily for signs of body movement, body weight, diarrhea and bloody stools. Bloody diarrhea events will be evaluated clinically by inspection of anal discharge, and a percentage value will be determined based on the number of animals with this condition at any given point of time. At the end of the experiment, rabbits will be sacrificed and the entire colon will be excised and placed on an ice plate and cleaned of fat and mesentery. The length of each colon specimen will be measured. The distal and proximal colons will be taken and will be fixed at 10% (w/v) buffered formalin for 24h at room temperature and embedded in paraffin and will be stained with haematoxylin–eosin for histological evaluation.

Preparations for Tissue Antioxidants Measurements

A full wall sections of the colon will be used, and 50 mg samples will be homogenized in sucrose buffer (0.25 M sucrose, 10 mM Tris, and 1 mM EDTA, pH 7.4). Tissue homogenates will be centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant will be used for determination of total glutathione using (GSH) a GSH determination kit (Sigma-Aldrich Chemicals GmbH, Bushs, Switzerland). The absorbance at 450 nm will be recorded and GSH activity will be calculated using the manufacturer's protocol.

Reverse Transcription and Quantitative Real-Time PCR Analysis

Total RNA will be isolated from the colon homogenate using the RNeasy mini kit (Qiagen, Hilden, Germany). The concentration will be determined using a UV spectrophotometer (Shimadzu, Japan). RNA (1 µg) will be used as the template for reverse transcriptase (RT) reactions using an oligo-(dT)15 primer (Promega, Madison, WI). The quantitative real-time PCR (qRT-PCR) reactions will be performed with the StratageneMx3000P qPCR system (Stratagene, La Jolla, CA), and commercially available SYBR Green PCR Master Mix reagent kit will be used for quantification of gene expression. Rabbit-specific primers will be designed for the genes of interest (Table 1). The housekeeping gene β -actin will be used as an internal control, and quantification of the transcripts will be performed by the $\{\Delta\Delta\Delta\}$ CT method [30].

Table (1). Sequences of primers and probes of quantitative RT-PCR Primer (the primers were chosen from Pubmed database).

No.	Genes	Forward primer	Reverse primer
1	<i>IL-1b</i>	5'-TCCTCTGTGACTCGTGGGAT-3'	5'-TCAGACAGCACGAGGCATTT-3'
2	<i>NF-kB/p65(RelA)</i>	5'-CCACGATCTGTTTCCCTCAT-3'	5'-TGATCTCCACATATGGCCCAG-3'
3	<i>P38</i>	5'-AGTGGCTGACCCCTTATGAC-3'	5'-CACAGTGAAGTGGGATGGA-3'
4	<i>Bax</i>	5'-CTGCAGAGGATGATTGCTGA-3'	5'-GATCAGCTCGGGCACTTTAG-3'
5	<i>Bcl-2</i>	5'-GCTACGAGTGGGATACTGG-3'	5'-GTGTGCAGATGCCGGTTCA-3'
6	<i>βactin</i>	5'-TCGTGCGTGACATTAAAGAG-3'	5'-ATTGCCGATAGTGATGACCT-3'

Statistical Analysis

Data will be expressed as mean \pm standard error of means (SEM). Comparisons will be performed by one-way ANOVA followed by post hoc Dunnett's test when applicable. Comparison between positive and negative control groups will be performed by t-Student test. The histopathological damage score of the groups will be compared by the Kruskal-Wallis and Mann-Whitney U tests.

Expected outcomes & Conclusion

The final goal of this project is to use the information obtained to design the new treatments developed from plant scale to produce a potent therapeutic preparation in treatment of Rabbit Colitis. Likewise, based on the optimization in the laboratory-scale test, and commercial-scale trials to be manufactured locally and marketed after making stages rely followed. ■

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