EVALUATION OF ANTI-HEMORRHOIDAL ACTIVITY OF "TRI 01" HARD CAPSULE ON EXPERIMENTAL MODEL OF HEMORRHOIDS IN WISTAR IN RATS

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This study evaluated the anti-hemorrhoidal effect of "TRI 01" hard capsule on croton oil-induced hemorrhoids model in rats. Hemorrhoids were induced by applying 6% croton oil preparation in the ano-rectal region. Then, rats were randomly assigned to groups. For the two "TRI 01" groups, "TRI 01" was orally administered at doses of 2.64 and 7.92 g/kg per day for seven days, respectively. Daflon (360 mg/kg per day) was used as reference anti-hemorrhoidal drug. Weighting of ano-rectal tissue, calculation of ano-rectal coefficient, and histology of ano-rectal tissue were conducted two hours after the last administration on the seventh day. The results indicated that treatment using "TRI 01" hard capsule significantly decreased the weight of ano-rectal tissue and the rectoanal coefficient compared to the vehicle-treated hemorrhoid group. Histological observation revealed that TRI 01-treated animals had lesser degree of inflammatory cells, degenerative changes congestion, edema along with lesser degree of necrosis in the mucosal epithelium of ano-rectal tissue as compared to croton-induced hemorrhoid group. Our study demonstrated that "TRI 01" at doses of 2.64 and 7.92 g/kg had anti-hemorrhoidal activity on croton oil-induced hemorrhoids model in rats.

Keywords: TRI 01, experimental hemorrhoid, croton oil.

I. INTRODUCTION

Hemorrhoids, one of the most common gastrointestinal diseases with the highest world wide incidence, is defined as the symptomatic enlargement and abnormally downward displacement of anal cushions. Hemorrhoids affect millions of people around the world, and represent a major medical and socioeconomic problem.[1,2] Hemorrhoids develop due to increase in pressure on the veins of the rectal region, which causes abnormal dilatation and distortion of the vascular channel, leading to the extravasation of blood around the perianal and anal vein. Hemorrhoids usually present with itching, rectal pain, or rectal bleeding. Patients with hemorrhoidal disease may experience any of the following symptoms: bleeding, a painful anal mass, swelling, discomfort, discharge, hygiene problems, soiling, and pruritus.[3-6] Treatment of symptomatic hemorrhoids ranges from dietary advice, lifestyle modification, and pharmacological approaches to office-based procedure and radical surgery depending on their grade and severity.[7] However, treatment of hemorrhoids in modern medicine is still in its infancy.[6] Due to limited modern pharmacotherapeutic options available for treatment, herbal medicines can be a viable

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choice of therapy. In the literature, there are few studies reporting the experimental evaluation of medicinal plants or their products in animal models of hemorrhoids.

Applying croton oil to the ano-rectal region leads to severe vasodilatation and subsequent inflammation, congestion, and edema, all of which are characteristics of hemorrhoids.[8] Thus, the croton oil model is useful for evaluating the effect of anti-hemorrhoidal drugs.[8-10] "TRI 01" hard capsule, a polyherbal formulation, is a drug aimed to treat hemorrhoids. To date, there are no systematic scientific studies to evaluate its effectiveness in experimental models of hemorrhoids. Experimental evaluation of the effect of the herbal medicine is necessary to develop further study in clinical settings. Therefore, the present study aims to demonstrate the anti-hemorrhoidal effect of "TRI 01" hard capsule in rats with hemorrhoid induced by croton oil.

II. MATERIALS AND METHODS

1. Preparation of "TRI 01" hard capsule

A "TRI 01" hard capsule consists of a mixture of the following medicinal herbs: *Radix Codonopsis pilosula* 0.35g, *Pericarpium Citri Reticulatae* 0.2g, *Spathodea campanulata* 0.35g, *Cimicifuga foetida* 0.2g, *Bupleurum chinesnis* 0.2g, *Angelica sinensis* 0.2g, *Glycyrrhiza uralensis* 0.1g, *Atractylodes macrocephala* 0.2g, *Semen Nelumbinis* 0.2g, and *Semen Coicis* 0.2g. The "TRI 01" hard capsule is provided by Traphaco High Tech Joint Stock Company according to the principles of Good Manufacturing Practice (GMP), Good Laboratory Practice (GLP) and Good Storage Practice (GSP). The expected dosage in clinical setting is ten hard capsules per day. This hard capsule is dissolved in water before oral administration.

2. Experimental animals

Healthy adult *Wistar* rats (aged 8 - 10 weeks and weighed 200 - 220g) were used for the study. Rats were housed in the laboratory animal room at 25 ± 1ºC, under 65 ± 5% humidity and 12h dark-light cycle (from 7:00 - 19:00)). Commercial laboratory food and tap water were given *ad libitum*. Rats were kept in these housing conditions for one week to acclimatize before starting the experiment.

3. Methods

Grouping and treatments

The rats were divided into five groups of ten animals as follows:

- Group 1 (normal control group): received 10 ml/kg per day sterile distilled water.

- Group 2 (croton oil-induced hemorrhoid): received vehicle (sterile distilled water) 10 ml/kg per day.

- Group 3 (croton oil-induced hemorrhoid + Daflon): was orally administered Daflon® (*Les Laboratoires Servier Industrie, France*) at dose 360 mg/kg per day.

- Group 4 (croton oil-induced hemorrhoid+TRI 01): was orally administered "TRI 01" at dose 2.64 g/kg per day (*equivalent to human recommended dose, conversion ratio 6*).

- Group 5 (croton oil-induced hemorrhoid+TRI 01): was orally administered "TRI 01" at dose 7.92 g/kg per day (*3 times as high as human recommended dose*).

Induction of hemorrhoids

Hemorrhoids were induced in rats as described previously.[9,10] Hemorrhoids were induced in all groups, except normal control group, by applying croton oil preparation. Croton oil mixture was prepared using deionized water, pyridine, diethyl ether, and 6% croton oil (Sigma Aldrich, St. Louis, USA) in diethyl ether in the ratio of 1:4:5:10. Rats were fasted overnight

before application of croton oil preparation. Sterile cotton swabs (4mm diameter) soaked in 100μl of croton oil preparation were inserted into the anus (ano-rectal portion, 20mm from anal opening) of all the study animals and kept for 30 seconds. A linear development of edema was observed up to seven to eight hours after the croton oil preparation application.[9,10]

Assessments

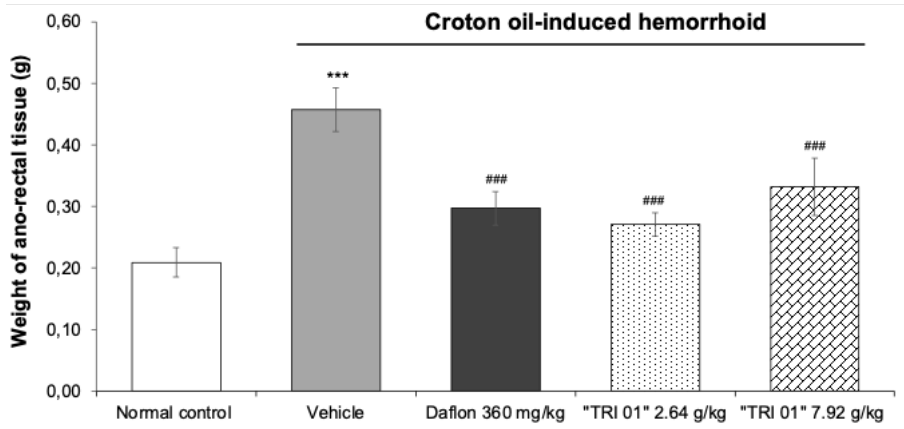
Twenty-four hours after induction, all animals were given drug treatments for seven days. On day seven, two hours after the last administration, the animals were sacrificed and the ano-rectal tissue specimens of 20 mm in length were dissected and weighed. The tissues were mounted on a plain paper and the inflammation was noted. The rectoanal coefficient (RAC) was calculated using the formula: RAC = (weight of ano-rectal tissue (mg))/body weight (g). At the end of the experiment, 30% of rats of the each group were dissected to evaluate the anorectal micro-structure. A small portion of ano-rectal tissue was fixed in 10% formaldehyde solution for histology studies. The tissue was further processed by conventional method to obtain thin sections of 5µm thickness which were subsequently stained with hematoxylin and eosin. The slides were examined under microscope for pathological changes. Histological observation of the rectoanal tissue was done to note the appearance of inflammatory cells, degenerative changes congestion, edema along with degrees of necrosis in the mucosal epithelium of ano-rectal tissue.

4. Statistical analysis

SigmaPlot 12.0 (SYSTA Software Inc, Richmond, CA, USA) was used for statistical analysis. Values were presented as mean ± standard deviation. Data were analyzed by a one-way ANOVA followed by post hoc Student-Newman-Keuls test for multiple comparisons. P values < 0.05 were considered statistically significant.

III. RESULTS

Figure 1 showed increases in the weight of ano-rectal tissue in vehicle-treated hemorrhoid group compared to normal control group ($p < 0.001$). Treatment with “TRI 01” hard capsule at doses of 2.64 and 7.92 g/kg per day and with Daflon at dose of 360 mg/kg per day significantly decreased the weight of ano-rectal tissue compared to the model group ($p < 0.001$). There was no significant difference in the weight of ano-rectal tissue between the two TRI 01 groups.

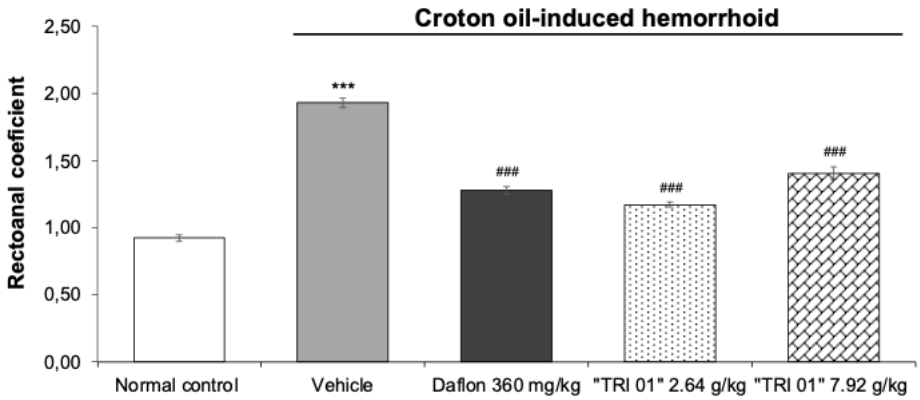


**p < 0.05; **p < 0.01, ***p < 0.001: compared to the normal control group*

#p < 0.05, ##p < 0.01, ###p < 0.001: compared to vehicle-treated croton oil-induced hemorrhoid model in rats

Figure 1. Effect of "TRI 01" hard capsule on the weight of ano-rectal tissue (n = 10)

Each data column represents the mean ± SD



**p < 0.05; **p < 0.01, ***p < 0.001: compared to the normal control group*

#p < 0.05, ##p < 0.01, ###p < 0.001: compared to vehicle-treated croton oil-induced hemorrhoid model in rats

Figure 2. Effect of "TRI 01" hard capsule on the ano-rectal coefficient (n = 10)

Each data column represents the mean ± SD

Figure 2 showed that, compared to the normal control group, croton oil preparation treatment increased the rectoanal coefficient in vehicle-treated hemorrhoid group ($p < 0.001$). Administration of "TRI 01" hard capsule at doses of 2.64 and 7.92 g/kg per day and Daflon at dose of 360 mg/kg per day caused significant reduction in the rectoanal coefficient compared to the model group ($p < 0.001$). There was no significant difference in ano-rectal coefficient between the two TRI 01 groups.

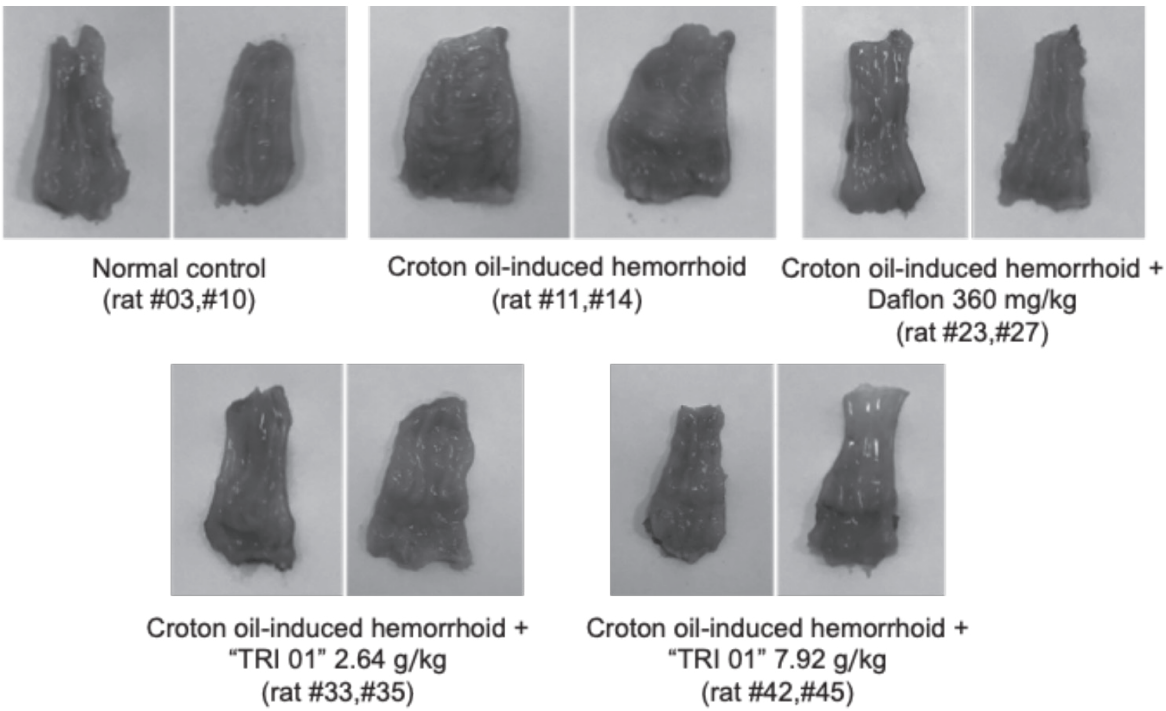


Image 1. Effect of TRI 01 hard capsule on macroscopic image of rectoanal tissue in a rat model of croton oil-induced hemorrhoids

On the macroscopic image, the isolated rectoanal tissue of normal control rat was observed to be cylindrial, with rectal contraction into segments, light color, and no stagnant stools. Croton oil preparation induced severe inflammation, edema, congestion, resulted in rectum size much larger than that of the normal control group. In Daflon and TRI 01-treated groups, the degree of inflammation and edema decreased compared to the model group. The reduction of fecal retention was observed. After for seven days of treatments, rectum size of rats in the Daflon and TRI 01 groups was smaller compared to the model group.

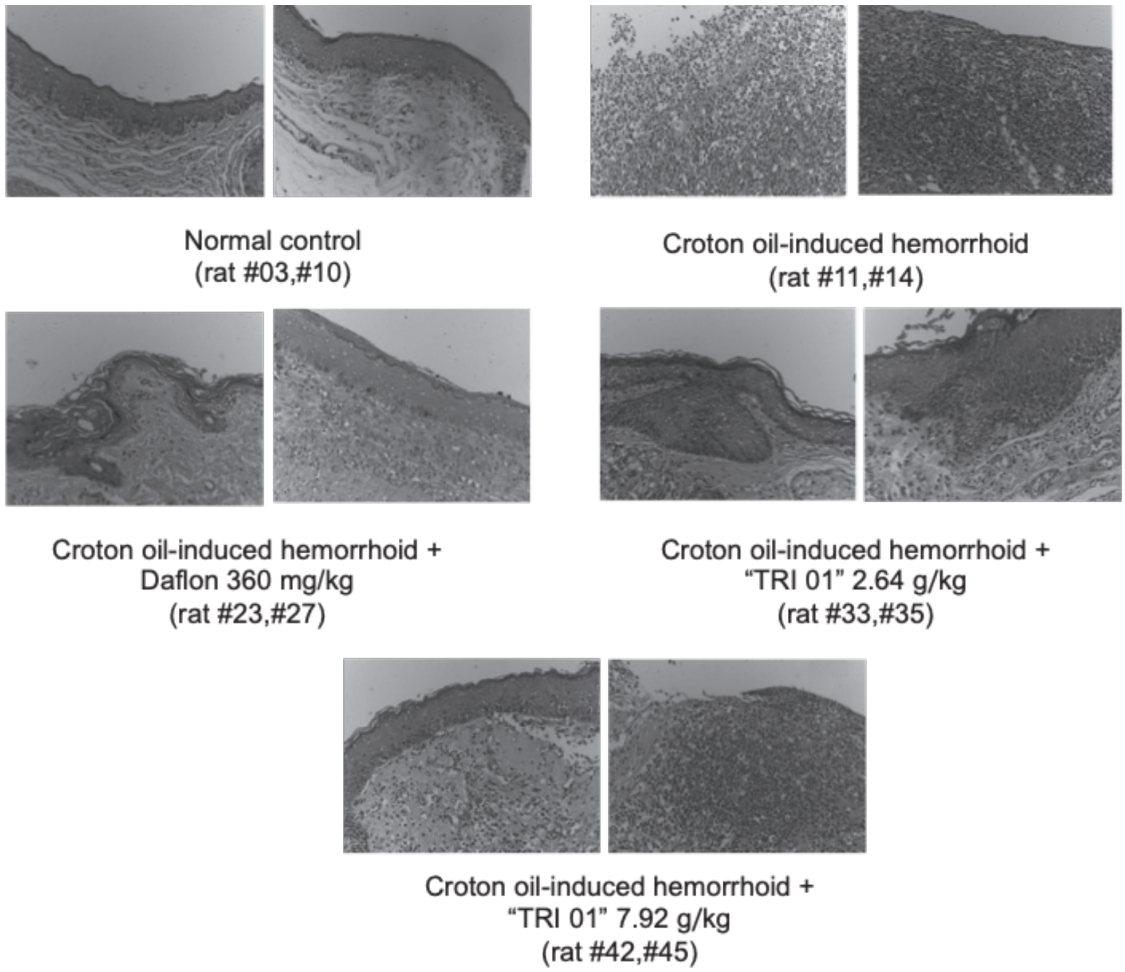


Image 2. Effect of TRI 01 hard capsule on histology of rectoanal tissue in a rat model of croton oil-induced hemorrhoids

The normal control rats showed normal appearance and architecture. The structure of the epithelial layer, the glandular layer, the muscularis submucosa were not abnormal. In vehicle-treated hemorrhoid group, rectoanal tissue marked to severe inflammation, and degeneration and necrosis were observed. The results showed that, compared to croton-induced hemorrhoid group, Daflon and TRI 01-treated animals had lesser degree of inflammatory cells, degenerative changes, congestion in the mucosal epithelium in ano-rectal tissue.

IV. DISCUSSION

Hemorrhoidal disease is one of the most common gastrointestinal diseases with a high prevalence.[1] In experimental study, the croton oil application in the ano-rectal region causes severe inflammation, a characteristic feature of hemorrhoids.[7] Croton oil application is known to cause migration of inflammatory cells and subsequently release of inflammatory lipid metabolites, kinins, nitric oxides, cytokines, along with increase in tissue lipid peroxidation and myeloperoxidase activity.[9] Our previous study showed that, compared to normal rats,

application of croton oil preparation with exposure for 30 seconds caused the significant increase of inflammatory cells, degenerative changes, and congestion in the mucosal epithelium in ano-rectal tissue.[10]

In this study, treatment with "TRI 01" at doses of 2.64 and 7.92 g/kg per day for seven days significantly ameliorated hemorrhoidal parameters suggesting the curative effect of this capsule. "TRI 01" significantly decreased the weight of ano-rectal tissue and the rectoanal coefficient compared to the model group. These findings were further supported by histopathological observations where "TRI 01"-treated animals showed lesser degree of inflammatory cells, congestion, edema, and necrosis of the mucosal cells, compared to model rats. There was no significant difference in the weight of ano-rectal tissue and ano-rectal coefficient between two doses. To our knowledge, Radix *Astragali membranacei*, Radix *Astragali membranacei*, *Rhizoma Cimicifugae*, Radix *Angelica sinensis*, and *Semen Coicis* were components in a traditional Chinese medicine formulation for treating hemorrhoid. Patients with mild hemorrhoid can be cured by orally taking the medicine for 1 to 2 times per day for 3 to 5 days. Patients with severe hemorrhoid can be cured after the medicine is simultaneously taken orally and used externally for 3 to 5 days. A cure rate of various kinds of hemorrhoid is 100%. The medicine has the advantages of being simple and convenient to prepare and to take, with no stimulation and no pain.[11] In addition, roots of *Codonopsis pilosula*, *Pericarpium Citri Reticulatae*, *Spathodea campanulata*, *Angelica sinensis*, and *Atractylodes macrocephala* also exerts anti-inflammatory activity.[12-16] This effect contributes to the treatment of hemorrhoids. In summary, the findings of this study indicated "TRI 01" hard capsule showed some beneficial effects on croton oil-induced hemorrhoids and validates its ethnomedicinal use in treatment of piles.

V. CONCLUSION

The present study demonstrated that "TRI 01" hard capsule at doses of 2.64 and 7.92 g/kg per day had some anti-hemorrhoidal effects on croton oil-induced hemorrhoids in rats. Compared to vehicle-treated hemorrhoid group, "TRI 01" groups had significantly lower weight of ano-rectal tissue and the rectoanal coefficient, and had lesser degree of inflammatory cells, degenerative changes, edema, and necrosis in the mucosal epithelium of ano-rectal tissue. There was no significant difference in weight of ano-rectal tissue and the rectoanal coefficient between two "TRI 01" groups.

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