Anti-Hemorrhoidal Activity of *Pluchea indica* Leaves Aqueous Extract in Croton Oil-Induced Hemorrhoids in Experimental Animals

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**Background and Objective:** *Pluchea indica* (L.) Less ("Khlu" in Thai) is traditionally used as a folk medicine for the treatment of many diseases and *Pluchea indica* leaves tea is quite famous and used to help cure hemorrhoids without any scientific evidences. This study focused on the investigation of the effects of aqueous extract of *Pluchea indica* leaves on the movement of gastrointestinal tract in mice and the croton oil-induced hemorrhoid in rats.

**Methods:** The effects of *Pluchea indica* leaves aqueous extract (PIE) at the doses of 50, 100 or 150 mg/kg on whole gut transit time and intestinal transit rate in male ICR mice were tested using the charcoal test. Male Wistar rats were forced fed with PIE at 50 mg/kg/day once daily for 10 consecutive days. On day 5, the animals were induced to be hemorrhoids by inserted cotton bud swab soaked with cotton oil preparation (100 μl) into the anus. Rats were killed on day 11, anus and spleens are removed and weighed and macroscopic changes of the rectum were observed.

**Results:** Oral administration of PIE at either 50, 100 or 150 mg/kg had no effect on whole gut transit time or intestinal transit rate in mice. PIE at 50 mg/kg/day could significantly attenuate the severity of the croton oil-induced hemorrhoids in rats as seen by a significant reduction of spleen and rectum weights and the severity of the rectal damage was significantly reduced.

**Conclusions:** The result of this study supported the effectiveness of the traditional use of *Pluchea indica* leaves tea in the treatment of hemorrhoids. However, at the doses tested, no effect on the gastrointestinal
Introduction

Hemorrhoids are the most common cause of bleeding per rectum and are responsible for considerable patient suffering and disability. The main symptoms of hemorrhoids are bleeding, itching, soiling, and pain with swollen veins in the areas around anus and lower rectum, similar to varicose veins. Occasionally, a clot may form in a hemorrhoid and can be extremely painful. Hemorrhoid disease has a high impact on quality of life and can be managed with a multitude of surgical and nonsurgical treatments. Generally, nonsurgical treatments such as medical management, dietary modifications and behavioral therapies are the mainstays of initial hemorrhoid therapy. Although the exact cause of symptomatic hemorrhoids is still unknown, a number of factors are believed to play a role, including irregular bowel habits (constipation or diarrhea), lack of exercise, nutritional factors (low-fiber diets), increased intra-abdominal pressure (prolonged straining, ascites, an intra-abdominal mass, or pregnancy), genetics, anal intercourse, an absence of valves within the hemorrhoidal veins, and aging\(^1\). Presently, oxidative stress is believed to have an important role in the pathogenesis of various diseases including hemorrhoids. It has been shown that many herbs accomplish haemorrhoid therapy by inhibiting lipid peroxidation and plugging up minute leaks and holes in the veins and capillaries thereby promoting vein elasticity and acting as vasoconstrictors in the perianal area\(^2,3\).

The leaves of *Pluchea indica* has been shown to have many health-promoting compounds, such as, dietary fiber, calcium, beta-carotene, chlorogenic acid, caffeic acid and quercetin and has been commercially available in Thailand as a health-promoting drink in a form of tea\(^4\). To date, many evidences have been shown that the extracts of Khlu leaves have good antioxidant activity\(^5,6\). In addition, Khlu leaves extract also possess significant anti-inflammatory effect\(^7\) and inhibiting lipids and carbohydrate accumulation in adipocytes\(^8\). This study aimed to investigate the effects of the PIE on the movement of gastrointestinal tract (whole gut transit time and intestinal transit rate) in mice and the croton oil-induced hemorrhoid in rats.

Methods

**Animals:** Male ICR mice (weighing 20-40g) and male Wistar rats (weighing 200-400g) were used. Animals were housed in stainless steel cages and maintained (12h light/dark cycle) in a temperature of 36±2°C and fed with a standard chow diet and drinking water ad libitum. All procedures were complied with the standards for the care and use of experimental animals and approved by the Animal Ethics Committee of Khon Kaen University, based on the Animal Experimentation of National Research Council of Thailand with record No. 0514.1.75/81.

**Plant Extraction:** *Pluchea indica* leaves tea was purchased from Baan Khounthain, Thailand. *Pluchea indica* extract (PIE) was prepared by soaking the tea with hot water (~80°C) (10g/100ml) for 5 minutes, and the filtrate is filtered through multiple layers of gauze and cotton wool. The extraction procedure was repeated three times and the filtrate was collected and lyophilized with freeze dryer with percentage yield of 3.59. The PIE was stored in a sealed dark bottle, kept at 4°C and freshly dissolved in the distilled water before used. The dose of PI extract is expressed as the weight of PIE.

**Effects of PIE on whole gut transit time in mice:** Mice were fasted overnight (18h) before the experiments and divided into 4 groups (6 animals/group). Distilled water (control) or various doses of PIE (50, 100 or 150 mg/kg) were administered orally. Thirty minutes after, animals were forced fed with activated charcoal suspension (12g charcoal and 2g gum acacia were ground in a mortar and suspended in 130ml distilled water) at the dose of 0.1ml/10g body weight as a marker. The time taken for excretion of the marker was recorded as a whole gut transit time.
Effects of PIE on intestinal transit rate in mice: Mice were fasted overnight (18h) before the experiments and divided into 4 groups (6 animals/group). Distilled water (control) or various doses of PIE (50, 100 or 150 mg/kg) were administered orally. Thirty minutes after, animals were force fed with activated charcoal suspension (12g charcoal and 2g gum acacia were ground in a mortar and suspended in 130 ml distilled water) at the dose of 0.1ml/10g body weight as a marker. Thirty minutes after receiving the activated charcoal suspension, mice were killed by cervical dislocation and the small intestines were removed. The distance traveled by the charcoal head from the pylorus as well as the total length of the small intestine were measured and the intestinal transit rate was calculated as follow.

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\text{Intestinal transit rate (\%)} = \frac{\text{Length traversed by charcoal marker}}{\text{Total length of small intestine}} \times 100
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Effects of PIE on croton oil-induced hemorrhoids in rats: Rats were randomly divided into four groups (6 animals/group). On each day, rats were force fed with distilled water (control) or PIE (50mg/kg/day) once daily for 10 consecutive days. On day 5, after feeding, hemorrhoids were induced by applying 100μl of croton-oil preparation (deionized water, pyridine, diethyl ether, and 6% croton oil in diethyl ether in the ratio of 1: 4: 5: 10) on cotton bud, inserted into the anus (about 2cm) and kept for 60 seconds. On each day of the experiment, the body weights of rats were determined. On day 11, all rats were killed by diethyl ether and specimens of rectoanal portion and spleens were removed and weighed. The macroscopic change of the rectoanus was investigated.

Discussion

This study showed that PIE at the dose of 50mg/kg could significantly reduce the severity of croton oil-induced hemorrhoids without any effect on gastrointestinal movement. Many bioflavonoids have been shown to have efficacy in the treatment of hemorrhoids and they exhibit phlebotonic activity, vasculoprotective effects, and antagonism of the biochemical mediators of inflammation. Khlu leaves have been reported to have good antioxidant and anti-inflammatory activities by inhibition of NO production and INOS suppression via the suppression of NF-κB activation. This might explain the...
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**Figure 2** Gross structure of rat’s rectoanal tissue. Control rats (A) showed normal appearance, while rats received croton oil (B) showed severe inflammation. Rats pretreated with PIE before treated with croton oil (C) showed minimal inflammation.

**Figure 3** Effects of PIE on the body weight (A), spleen weight (B) and rectoanus weight/body weight (C) of croton oil-induced hemorrhoid rats.

The anti-hemorrhoidal activity of PIE as observed in this study. However, at the dose tested, no effect of PIE on gastrointestinal movement was observed, thus, at this dose no effect on reducing constipation could be claimed. It is interesting to further investigate whether other mechanisms may contribute to the anti-hemorrhoidal effect of **Pluchea indica** leaves.

**Conclusion**

The result of this study supported the effectiveness of the traditional use of **Pluchea indica** leaves tea in the treatment of hemorrhoids. However, at the dose tested, no effect on the gastrointestinal movement was seen.

**Acknowledgements**

MS. Koneouma Senvorasinh is a M.Sc. student in Pharmacology Department and partly supported from Faculty of Medicine, Khon Kaen University and Government of the Lao People’s Democratic Republic of Lao PDR.

**References**


