ผลของสารสกัดเอธิลอะซิเตทจากต้นหญ้ายางต่อการหดตัวของลำไส้หนูขาว

Effect of Ethyl Acetate Extracts of *Euphorbia heterophylla* Linn on Rat Ileum Contraction

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บทคัดย่อ

ใบหญ้ายางนิยมใช้เป็นยาระบายในประเทศไทย การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ของสารสกัดจากใบหญ้ายางต่อการเคลื่อนไหวของกล้ามเนื้อเรียบที่ลำไส้เล็กและกลไกการออกฤทธิ์ โดยนำสารสกัดใบหญ้ายางสดด้วยเมธานอล ชั้นเอธิลอะซิเตทและชั้นน้ำที่ได้จากการสกัดด้วยเอธิลอะซิเตทมาศึกษาผลต่อการเคลื่อนที่ของลำไส้เล็กที่แยกออกจากร่างกายหนูทดลอง จากผลการทดลองพบว่าสารสกัดเมธานอลและชั้นเอธิลอะซิเตทของใบหญ้ายางไม่เกิดการหดตัวของลำไส้เล็กหนู ส่วนสารสกัดชั้นน้ำสามารถทำให้ลำไส้เล็กของหนูหดตัวเพิ่มขึ้นและแปรผันตามปริมาณของสารสกัดชั้นน้ำที่ใช้ ผลของสารสกัดต่อการเคลื่อนไหวของลำไส้เล็กของหนูมีฤทธิ์คล้ายคลึงกับอะเซทธิลคลอรีน ฮิสตามีน และโพแทสเซียมคลอไรด์ และมีลักษณะขึ้นกับความเข้มข้นสาร นอกจากนี้ฤทธิ์ดังกล่าวถูกยับยั้งได้ด้วยอะโทรปีน และเวอราปามิล แต่ไม่ถูกยับยั้งด้วยคลอเฟนิรามีน จึงเป็นไปได้ที่การออกฤทธิ์ของสารสกัดใบหญ้ายางชั้นน้ำจากการสกัดด้วยเอธิลอะซิเตทต่อการหดตัวของลำไส้เล็กของหนูจะผ่านทางตัวรับมัสคารินิกและช่องทางเข้าเซลล์ของแคลเซียม การออกฤทธิ์ดังกล่าวจึงมีประโยชน์ในแง่การใช้ใบหญ้ายางเป็นยาระบาย

คำสำคัญ : ต้นหญ้ายาง ลำไส้ การหดตัว ยาระบาย

Abstract

Euphorbia heterophylla Linn. leaves are frequently used as a laxative in Thailand. The purposes of this investigation were to study the pharmacological effect of *Euphorbia heterophylla* Linn. on the motility of intestinal smooth muscle and its mechanisms of action. The methanolic, ethyl acetate and the aqueous fractions from the fresh ethyl acetate extract of the plant were tested for their effect on the motility of isolated rat ileum. Neither the methanolic nor the ethyl acetate fractions produced a contraction of the ileum, whereas the aqueous fraction had contractile effect on the ileum. The effect was similar to that of acetylcholine, histamine and potassium chloride and was dose-related. The increase in motility was inhibited by atropine and verapramil, but not by chlorpheniramine. Therefore, the extract might act on the intestinal smooth muscle mainly through muscarinic receptors and perhaps calcium channels. The results on the isolated rat ileum supported the use of this plant as a laxative in Thai folk medicine.

Key words : *Euphorbia heterophylla*, ileum, contraction, laxative

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Introduction

Constipation is a gastrointestinal motility disorder, a common problem in both adults and children. Its worldwide prevalence ranges from 2.5% to 7.9% of the adult population and 0.7% to 29.6% in children [Mugie, 2011]. Using a laxative is the most common way to relieve constipation. Laxatives can act by increasing intestinal motility and /or intestinal secretions [Brunton, 2012]. *Euphorbia heterophylla* Linn. (EH) is a plant belongs to family Euphorbiaceae. It is formally known as "Yha-Yang" in Thailand and widely used for relieving constipation in the south of Thailand. It is distributed worldwide and has been used as a folk medicine. Its leaves are commonly used for its activity as an antioxidant, an anti-inflammatory and a laxative agent [Abbasi *et al*., 2013; Falodun *et al*., 2006; Falodun & Agbakmuru, 2004]. The phytochemicals responsible for the laxative activity of *E. heterophylla* leaves have been shown to be carbohydrates and phorbol ester [Falodun & Agbakmuru, 2004]. However, its mechanism as a laxative is not known. The aims of this study therefore were to screen extracts from *E. heterophylla* leaves for their ability to induce motility in rat ileum and to determine its mechanism of action as an anti–constipation agent.

Materials and Methods

Drugs and chemicals

Acetylcholine chloride (ACh), atropine sulphate, histamine bisphosphate monohydrate (His), chlorpheniramine maleate (CPM) and verapamil hydrochloride were obtained from Sigma Chemical Co., USA. Potassium chloride (KCl) was obtained from Merck, Darmstadt, Germany. All the solvents (methanol and ethyl acetate) were purchased from J.T. Baker, NJ, USA.

Plant Material and extraction

The fresh leaves of *E. heterophylla* were collected in December 2012 from Songkhla Province, Thailand. The voucher specimen (PSU NO. 00014513) was identified and authenticated by Assist. Prof. Dr. Charan Leeratiwong, Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

The fresh leaves of *E. heterophylla* were cut into small pieces and immersed in methanol and ethyl acetate for 72 h at room temperature. The methanol soluble fraction was filtered and then concentrated at 45^{o}C using a rotary evaporator to obtain the methanol crude extract. After immersion in ethyl acetate, the residue was partitioned between ethyl acetate and water to obtain ethyl acetate soluble and aqueous fractions. The ethyl acetate fraction was concentrated at 45^{o}C using a rotary evaporator. The aqueous fraction was frozen at -40^{o}C and dried for 48 h using a freeze dryer. The yield of the mathanolic, ethyl acetate fraction and aqueous fraction extracts were 2.29%, 0.69% and 2.71%, respectively.

Animals and Experimental procedures

Adult Wistar rats and guinea-pigs of either sex were supplied by the Sounthern Laboratory Animal facility, Prince of Songkla University, Hat Yai, Songkhla, Thailand. They were housed in the temperature controlled room at 25±2 °C with a 12 h light/dark cycle. The animals were fed with food and water *ad libitum*. All procedures were approved by the Ethics Committee on Animal Experiment (Ref.02/2012), Prince of Songkla University, Thailand.

The animals were fasted for 8 h before sacrifice by cervical dislocation and exsanguinations. The ileum above the ileoceacal junction was dissected. They were cut into 2-cm segments and lumen contents were expelled by gentle irrigation with a 37°C Kreb's solution using a Pasteur pipette. The ileum was then set up in an organ bath filled with 20 mL of Kreb's solution and aerated with 95% O_2 and 5% CO_2. The contractions of the ileum was monitored and recorded isometrically with a force FT03E displacement transducer connected to a Grass Model 7 polygraph (Grass International Co., Quincy, Mass, USA.).

The effect of extracts (0.06-2 mg/mL) was studied on the contraction of the guinea-pig and rat ileum. The contractile responses were compared with those produced by the standard spasmogens: acetylcholine ($5x10^{-9}$ M), histamine (10^{-8} g/mL) and potassium chloride (30 mM) and the inhibition of contractions by their corresponding antagonist: atropine ($2x10^{-7}$ M), chlorpheniramine ($8x10^{-9}$ g/mL) and verapamil (10^{-7} g/mL).

Statistical analysis

Quantitative data were expressed as a mean value±standard error of the mean (SEM) of three independent experiments. Differences between means were analyzed using the student's *t-test*. A probability of less than 0.05 was taken to indicate statistical significance.

Results

Effect of *E. heterophylla* extracts on the contraction of isolated rat ileum

The methanolic and ethyl acetate fraction extracts of *E. heterophylla* did not induce ileum contraction (data not shown) whereas the aqueous fraction obtained from ethyl acetate extract of *E. heterophylla* caused a dose-dependent increase in the force of contraction of the isolated rat ileum (Figure 1).

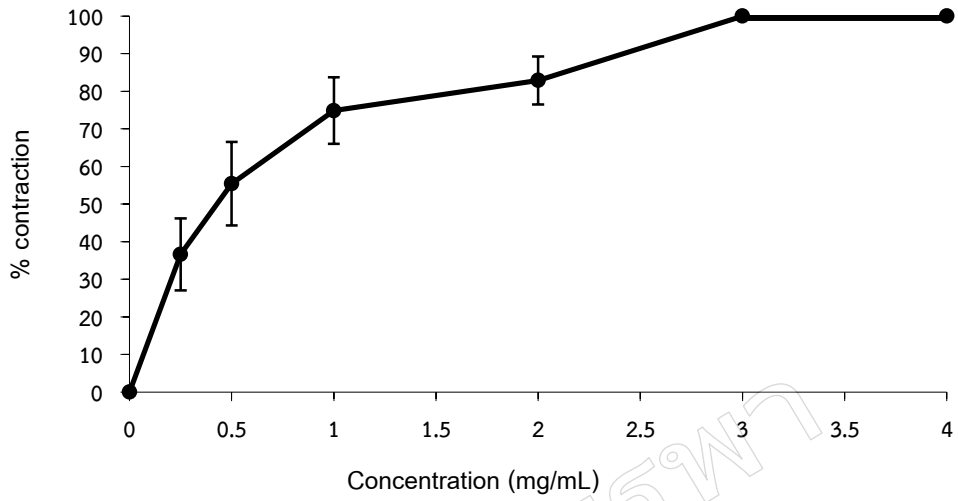

Figure 1 Effect of the aqueous fraction of ethyl acetate extract of *E. heterophylla* on contraction of isolated rat ileum

Effect of antagonists on the responses of isolated rat and guinea-pig ileum to the aqueous fraction of ethyl acetate extract of *E. heterophylla*

The contractions of isolated rat ileum induced by the aqueous fraction from *E. heterophylla* were completely inhibited by atropine ($2x10^{-7}$ M) at a dose that completely blocked the ACh ($5x10^{-9}$ M)-induced contraction. Whereas verapamil (10^{-7} g/mL) completely abolished the contraction of the ileum induced by KCl (30 mM), the contractions induced by the aqueous fraction from *E. heterophylla* were only partially inhibited by about 60-80% by verapamil at the same concentration. CPM ($8x10^{-9}$ g/mL) completely inhibited the contraction of the guinea-pig ileum produced by His (10^{-8} M). In contrast, it did not block the contraction produced by the active *E. heterophylla* fraction. The inhibitory effect of antagonists on the contraction of isolated rat and guinea-pigs ileum induced by *E. heterophylla* are shown in Figure 2.

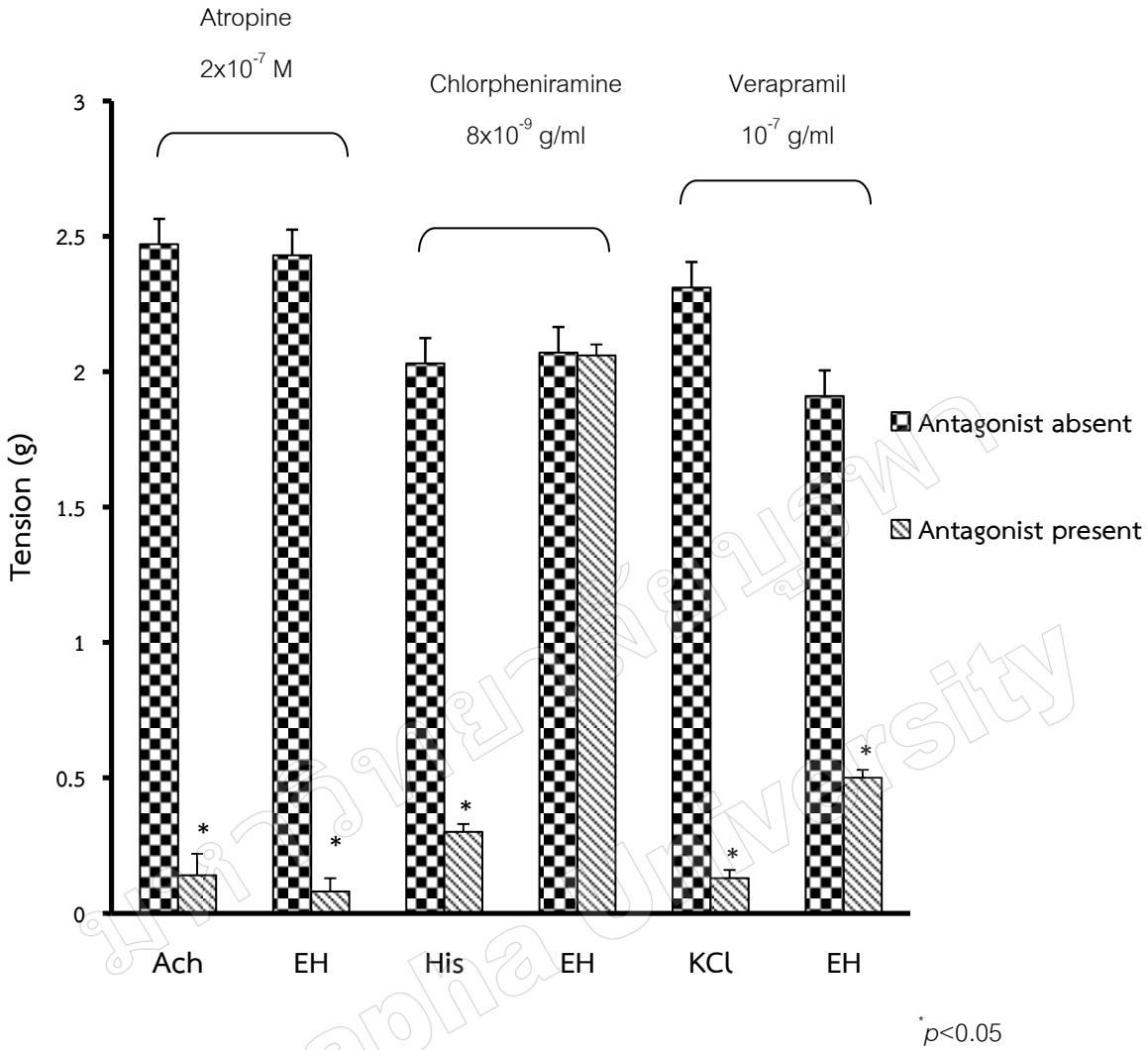

Figure 2 Inhibitory effect of antagonists on isolated ileum motility induced by *E. heterophylla* and other agonists. (ACh: acetylcholine, His: histamine, KCl: potassium chloride, EH: *E. heterophylla* extract).

Discussion and conclusion

The aqueous fraction of the fresh ethyl acetate extract of *E. heterophylla* produced a dose-related increase in the contraction of rat ileum similar to those of ACh, His and KCl. The present study demonstrated that the aqueous fraction of the fresh ethyl acetate extract from *E. heterophylla* had a contractile effect on the intestinal smooth muscle possibly through a similar mechanism to that of ACh and KCl.

ACh is a neurotransmitter agonist which can bind to muscarinic and nicotinic receptors and induce contraction of rat and guinea-pig ileums. The contraction mechanism of Ach is resulting in an increase of cytosolic calcium concentration [Unno *et al.*, 2003]. KCl can also produce ileum contraction by an increase of calcium

entry through voltage-operated calcium channel and myosin light chain phosphorylation [Ratz *et al*., 2005]. His was also found to induce contraction through H1 receptor in guinea-pig colon but not in rat colon [Aguilar *et al*., 2009]. After histamine binds to H1 receptor, the activation of phospholipase C induced increase in the intracellular calcium and evokes contraction of smooth muscle [Shahid *et al*, 2009]. Based on the results, the ileum of rats was used to study on the effect of ACh- and KCl-induced contraction whereas the His-induced contraction was used guinea-pig ileum similar to the previous study [Aguilar *et al*., 2009]. For the mechanism study of the extracts, we used a muscarinic receptor antagonist, a histaminic receptor antagonist and an L-type calcium channel blocker as standard drugs to identify the possible mechanism of action of *E. heterophylla* in inducing intestinal smooth muscle contraction. Our results showed that atropine, a muscarinic receptor antagonist, significantly inhibited the ACh and the *E. heterophylla* extract responses when compared with the same stimulants in the absence of antagonists. This clearly indicated that the extract might act on rat ileum through the muscarinic receptor pathway. Another probable mechanism that may cause an increase in the contraction of rat ileum was via activation of the L-type calcium channels. Varapramil, a calcium channel blocker, also significantly decreased the ileum contraction induced by both KCl and the extract. However, the effect of the extract on the guinea-pig ileum was only slightly inhibited by CPM, a histamic H_1 receptor antagonist, at a concentration that completely inhibited the effect of histamine. Therefore, we can imply that the contractive mechanism of *E. heterophylla* extract on ileum is different from that of histamine.

In conclusion, the results obtained from the isolated rat ileum supported the use in Thai traditional medicine of *E. heterophylla* as a laxative by increasing gastrointestinal motility. Before promoting its use, further investigation on any side effects and possible toxicities of the plant are required.

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