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Relaxant effects of *Azadirachta indica* A. Juss var. *siamensis* Valeton flower extract on isolated rat ileum contractions and the mechanisms of action

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ABSTRACT

Azadirachta indica A. Juss var. *siamensis* Valeton or commonly known as Siamese neem is one of the most well-known plant in traditional Ayurvedic medicine. The aim of the present study was to investigate the relaxant effects of *A. indica* on isolated rat ileum contractions and its potential underlying mechanisms involved. The isometric contractions of ileum segments were investigated in organ baths for spontaneous activity and response to aqueous extract of Siamese neem flower (SNF). The spasmolytic action of the extract was also assessed on contraction induced by acetylcholine and high potassium. Our findings indicate that cumulative concentrations of SNF aqueous extract induced relaxant effect on spontaneous rat ileum contractions. The extract has also suppressed the cumulative concentration response curve for acetylcholine and pottasium ions-induced contraction. The presence and absence of propranol (antagonist of β-adrenergic receptor) and L-Name (antagonist of nitric oxide synthase) in SNF aqeous extract co-treatment demonstrated no significant different in term of contraction activity when compared to SNF extract treatment alone. The treatment of SNF extract caused a significant inhibition in tissue contraction stimulated by accumulation of calcium ions. Our results showed the relaxant effect of SNF aqueous extract on the isolated rat ileum. In short, the SNF aqueous extract exhibited an inhibitory effect on the spontaneous ileum contactions particularly on the contraction stimulated by acetylcholine and high potassium. The observed effect might acted through the modulation of calcium channels. This findings provide a pharmacological basis for the traditional use of SNF for the treatment of gastrointestinal spasms.

1. Introduction

Gastrointestinal (GI) diseases and symptoms are known to cause an effect on quality of life, work and activity impairments, and health care costs.[1] A disturbance in coordination between the motor, sensory or secretory function of GI tract are known to cause GI functional and motility disorders.[2] Although the etiology of some cases of GI functional and motility disorders are still remain unclear, the common observed symptoms are including nausea, vomiting, bloating, abdominal discomfort or pain, constipation or diarrhea.[2] The onset of GI motility disorders has been attributed to several causes, which including the consequence complications of other systemic illnesses, such as diabetes.[2,3]

Generally, the pharmacological interventions for GI motility disorders are involved in altering the regulation of GI smooth muscle, enteric nervous system (ENS), autonomic ganglia, and central nervous system. Radulovic M et al. have suggested that release of neurotransmitters and several ion channels in GI tissues including ATP-sensitive potassium (K_{ATP}) channel, calcium ions

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(Ca^{2+})-activated potassium ions (K^+) channels, voltage-sensitive Ca^{2+} channels and chloride ion channels play an important role in regulating GI motility.[4] In fact, those mechanisms have served as the fundamental knowledge in new discoveries and development of new therapeutic agents. Cholinergic agonists or laxative agents are widely used for gastrointestinal motility disorders management.[4] However, these drugs have an undesirable side effect. Complementary and alternative medicines (CAM) have been associated with symptom management and quality of life in a common gastrointestinal disorder.[5] Therefore, the attention has been shifted to bio-prospecting the natural products to overcome or control GI motility disorders.

For centuries, herbs have been used in traditional medicine to treat GI disorders. With the recent advancement of technologies or scientific techniques, it enables the essences of this traditional knowledge to be explored further. These investigations have opened up more avenues which either allows a more systematic usage of traditional medicine or alternatively to be developed to become a standard drug. For instance, Siamese neem tree (*Azadirachta indica* A. Juss. var. *siamensis* Valeton) which is belong to Meliaceae family has been used in Ayurvedic medicine for more than four thousand years.[6] All parts of the neem tree (leaves, flowers, seeds, fruits, roots and bark) have been used for the treatment of inflammation, infections, fever, skin diseases and dental disorders.[7] The robustness and well-studied biological activities has thus contributed to its commercial exploitation for treatment of various diseases. In particular, it is traditionally suggested and used to treat diarrhea and peptic ulcer.[8–11] However, to the best of our knowledge the role of Siamese neem flower (SNF) on intestinal smooth muscle motility is remaining unclear. Therefore, the current study aims to study the effect of SNF extract on isolated rat ileum contractions induced by high potassium and acetylcholine, and investigated the possible mechanisms involved. The findings might help to explain the extensively usage of SNF for the treatment of gastrointestinal diseases.

2. Materials and methods

2.1. Chemicals

Atropine sulphate, propranolol, acetylcholine chloride and L-NAME were purchased from Sigma (USA), KCl, $CaCl_2$ and other reagents were obtained from Merck Company (Germany). The extract and chemicals were dissolved in distilled water for experimentation. The Krebs' solution used in these experiments had the following composition: 122 mM NaCl, 5 mM KCl, 10 mM HEPES, 0.5 mM KH_2PO_4, 0.5 mM NaH_2PO_4, 1 mM $MgCl_2$, 1.8 mM CaCl,[2] and 11 mM glucose; pH 7.3.

2.2. Plant materials

Siamese neem flowers (SNF) were collected from Tumbon BanMaetumboonyong Mueang Phayao, Thailand. The plant was identified by a botanist and a voucher specimen (No. 003805) was deposited at the herbarium of the Faculty of Biology, Naresuan University, Phitsanulok, Thailand.

2.3. Preparation of SNF aqueous extract

Siamese neem flowers were washed, cut in smaller pieces, and blended in an electric blender in distilled water (plants 100 g: water 300 mL). They were filtered to obtain a crude solution and subsequently freeze-dried by freeze dryer (Scam VacCoolSafe 110–4 Pro) until powder was obtained. The lyophilized sample powder or extract was then stored at −20 °C. Prior to experimentation, the powder extract was prepared by dissolving it in appropriate amount of distilled water.

2.4. Animals

Male Wistar rats (200–250 g) were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakhornpathom, Thailand. Experiments were approved by the Animal Ethics Committee of University of Phayao, Phayao, Thailand. Animals were maintained at Laboratory Animal Research Center, University of Phayao under 12-h dark/12-h light condition and free access to water and standard rodent diet.

2.5. Tissue preparation and experimental procedure

After animals were fasted overnight, they were deeply anesthetized by 50 mg/kg BW zolazepam/tiletamine (zoletil) and 3 mg/kg BW xylazine. Two pieces of ileum were isolated from 2 cm above the ileocaecal junction. The intraluminal content was flushed out with cooled Krebs' solution and cleaned off connective tissue surrounding. The tissue was mounted in an organ bath (30 mL) (the lower region was tightly tied to the bottom inside the bath while the upper region was connected to an isotonic force transducer) containing Krebs'solution and maintained at 37 °C, pH7.4, and a continuous supply of oxygen from air bubbles. The ileum was equilibrated for 1 h under 1 g resting and the solution was replaced every 15 min. Ileum contraction were measured using force transducer connected to an iWorx214 A/D converter (LabScribe2; Instruments, Thailand) and the data was recorded.

2.6. Relaxation effects of siamese neem flower

To examine the relaxation effect of SNF on ileum contraction, SNF extract (0.01–10 mg/mL) was cumulatively added into the organ bath once the contraction plateau was reached by the KCl (80 mM) induction. The contractions induced by the extract was recorded and normalized to KCl induced activity which was considered as the maximum contraction (100% contraction). In order to investigated the mechanism of action involved in relaxant effect demonstrated by SNF, experimentations with ileum preincubation in 100 μM L-Name (antagonist of nitric oxide synthase) for 20 min or 1 μM propanolol (antagonist of β-adrenergic receptor) for 30 min prior to KCl 80 mM exposure were then conducted. Following the preincubation period as stated above, the extract (5 mg/mL) was then added into the organ bath when KCl induced contraction reached a constant activity. On the other hand, the addition experiments were also conducted in order to examine the acetylcholine chloride (10^{-5} M, agonist of acetylcholine receptor) induced ileum contraction activity in either the presence or absence of extract (5 mg/mL).

2.7. Effect of siamese neem flower on extracellular Ca^{2+} influx

In order, to determine the role of SNF on extracellular calcium, Ileum was initially equlibrated in Ca^{2+} free Krebs' solution (0.01 mM EGTA, 122 mM NaCl, 5 mM KCl, 10 mM HEPES, 0.5 mM KH_2PO_4, 0.5 mM NaH_2PO_4, 1 mM $MgCl_2$, and 11 mM glucose; pH 7.3) for 30 min. Following the incubation, SNF extract was added into the solution. Subsequently, a cumulatively concentration of $CaCl_2$ (1–40 mM) was then added into the organ bath to stimulate the contraction. The contraction activity was observed and recorded for 10 min following the addition. The activity was determined by comparing the extract presence group to the control group or known as extract absence group.