

Spasmolytic Effect of Papaya (*Carica papaya* L.) Leave Alkaloid on Isolated Rat Myometrial Contraction *in vitro*

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Abstract

This study aimed to investigate the action of crude alkaloid extract from papaya leaves (PA) on the isolated rat myometrium *in vitro*. The alkaloids were extracted from papaya leaves using conventional acid-base method. The myometrium strips were taken from female Wistar rats pretreated with diethylstilbestrol (100 µg) for 24 h before the experiment. A preliminary experiment has shown that PA had no effect on myometrial contraction. However, PA (10^{-6} - 10^{-3} gm/mL) significantly inhibited myometrium contraction pre-contracted with a depolarizing solution in a concentration-dependent fashion. PA (10^{-5} , 3×10^{-5} or 10^{-4} gm/mL) or verapamil (10^{-9} , 10^{-8} and 10^{-7} M) were able to inhibit the myometrial contractile response to CaCl_2 (10^{-5} - 3×10^{-2} M) in Ca^{2+} -free solution and cause a rightward shift of log concentration-response curve. PA also significantly inhibited myometrium contraction produced by oxytocin (1 mU/mL), ACh (3×10^{-5} M) and $\text{PGF}_{2\alpha}$ (10^{-5} M). Furthermore, propranolol (10^{-7} M) caused no effect on uterine relaxation induced by PA (10^{-6} - 10^{-3} gm/mL), although it inhibited uterine relaxation induced by isoproterenol (10^{-10} - 10^{-5} M). These results indicated that PA was a uterine relaxant. Its mechanism of action may be unrelated to stimulation of β_2 -adrenergic receptors of the uterus. This action may be contributed to mainly the Ca^{2+} influx, possibly through voltage-operated Ca^{2+} channel on the plasma membrane, and to a lesser extent by a rise in Ca^{2+} release from internal storage. However, the action of PA at intracellular sites, downstream of Ca^{2+} influx such as the contractile machinery of the myometrium, cannot be excluded.

Keywords: *Carica papaya*, Papaya alkaloids, Uterine contraction, Uterine relaxation

Introduction

Papaya (*Carica papaya*, L., Caricaceae) is a common plant cultivated in tropical countries. It is easily cultivated and widely used as food or dessert [1]. Various parts of this plant (fruit, leaves, seeds or latex) contain many biologically active compounds and has been used as medicinal herb in many areas of the world. Recent studies revealed that the compounds exhibit various pharmacological properties such as promoting the function of digestive system, anthelmintic, anti-amoebic, anti-inflammatory, wound healing, antifertility and contraceptive, antifungal, antibacterial, antitumor, antioxidant, anti-dengue and antimalarial [1,2]

Papaya leaves are abundant of biologically active constituents such as alkaloids (carpaine, pseudocarpaine and dehydrocarpaine I and II), flavonoids (kaemferol and myricetin), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), carotene, caproside, vitamin C and vitamin E [2,3]. These constituents exhibit medicinal properties such as antibacterial, antiviral, antitumor, anti-inflammatory, antifertility, abortifacient, hypoglycemic, hepatoprotective and wound healing [3,4]. Papaya leaf extract also alleviated menstrual pain probably related to the reduction in blood level of prostaglandin $\text{F}_{2\alpha}$ in primary dysmenorrhea [5]. The latter effect could occur from one or more of its active constituents.

Carpaine is a principal alkaloid of papaya and present in various green parts of the plant [6]. Its actions were quite resembling to digitalis and emetine without most of their serious adverse effects. Carpaine and pseudocarpaine have been shown to have a depressive effect on the heart [7]. The uterus showed marked relaxation by this alkaloid [6]. More recent studies revealed antithrombocytopenic and antiplasmodial activity of this alkaloid which could be beneficial for the treatment of dengue fever and malaria [8,9].

Although, previous studies have demonstrated the pharmacological actions of carpaine, the mechanism of action of this alkaloid is poorly understood. In addition, carpaine is the most abundant alkaloid present in papaya leaves and possesses a uterine relaxing property. Papaya leaf extract have been shown its effect to relief menstrual pain. Carpaine and other alkaloids could work in concert with other active constituents of papaya leaf extract to ease the menstrual pain. This has led us to reinvestigate the effects of the alkaloid from papaya leaves, aiming to propose a mechanism of action of the alkaloid extract which has never been mentioned elsewhere.

Materials and methods

Preparation of plant materials

Papaya leaves (20 kg) were obtained from a papaya orchard in Ronpiboon District, Nakhon Si Thammarat Province, Thailand. They were examined by a botanist of Botany Section, Department of Biology, Prince of Songkla University. The leaves were cleansed and rinsed with distilled water, left to air-dry of excess water at ambient temperature in a well-ventilated room overnight. They were further dried at 50 °C until dryness in hot air oven. Leaf stalk and large veins were then separated from the green leaves and discarded. The remaining dried leaves were pulverized to powder (2.2 kg.) and used to extract alkaloids.

Drugs and Chemicals

Acetylcholine chloride, (\pm)-verapamil hydrochloride, DL-propranolol hydrochloride, (\pm)-isoproterenol hydrochloride, dimethylsulfoxide (DMSO) and diethylstilbestrol (DES) were obtained from Sigma Chemical Company. These chemicals, except DES were prepared as stock solutions in 0.1 % ascorbic acid in distilled water (acetylcholine 10^{-1} M, propranolol 10^{-4} M, verapamil 10^{-4} M, and isoproterenol 10^{-1} M). Diethylstilbestrol was prepared for intraperitoneal injection by dissolving in olive oil (1 mg/mL). Oxytocin (syntocinon) was purchased from Novartis and Pharmacia and PGF_{2 α} (Dinoprost tromethamine) from Upjohn (Belgium). Papaya leave's alkaloid (PA) was prepared as a stock solution (200 mg/mL) by dissolving in dimethylsulfoxide for use in all experiments. The stock solutions were diluted to appropriate concentrations with physiological solutions before adding into the organ bath. The total concentration of dimethylsulfoxide in the organ chamber after cumulative addition of PA stock solution was always less than 0.5 % and did not interfere with the contractile responses of the uterus. Sodium chloride (NaCl) and sodium bicarbonate (NaHCO₃) (Merck), potassium chloride (KCl), calcium chloride (CaCl₂) and glucose (Carlo Erba) were used for the preparation of physiological solutions, which were prepared freshly on the day of experiment.

Extraction procedures

Alkaloid extract from *Carica papaya* L. leaves was prepared as previously described [10]. In brief, the papaya leave powder (2.2 kg) was dampened thoroughly with concentrate ammonium hydroxide solution, and soaked in absolute methanol at room temperature for 3 days. This crude methanolic extract was then removed, filtered and stored at 4 °C. The extraction of the remaining leave powder with methanol was repeated until trace of alkaloid in the extracts as detected by Dragendroff's reagent showed negative results. All methanolic extracts from this step were then combined, and evaporated until dryness under reduced pressure at 50 °C using Rotavac Evaporator (Buchi®). The extract was then dissolved in aqueous sulfuric acid solution (2 % in distilled water), extracted with n-hexane to remove extractable materials such as fat materials and oils and the hexane fraction was discarded. The pH of aqueous fraction was then raised to pH 9 using ammonium hydroxide solution. This fraction was then extracted with chloroform in excess. The chloroform extracts were then combined and dry at 50 °C under reduced pressure. The crude alkaloid extract obtained from the last step was partially purified by dissolving in aqueous sulfuric acid solution (2 % sulfuric acid in water) and the alkaloid extraction procedure were repeated as described above. The crude alkaloid extracts (9.1 g) were obtained and stored in an air tight bottle at 4 °C until use.

Experimental animals

Female Wistar rats, age 2 - 3 months (weighing between 200 - 300 gm.) were obtained from Southern Animal Facility, Animal House Unit, Faculty of Science, Prince of Songkla University, Songkhla, Thailand. All animal protocols in this study were approved by the Institutional Animals Care and Use Committee, Prince of Songkla University (REF 24/51). They were housed in environmental

controlled-room (relative humidity 55 - 60 %, temperature 24 - 26 °C and 12 h light/dark cycle). The rats were given intraperitoneally with diethylstilbestrol (DES) (100 µg) 24 h before the experiment.

Experimental protocols

On the day of experiment, the rats were euthanized by cervical dislocation. Both sides of myometrium were removed and submerged in Jalon-Ringer solution. The myometrial strips were cleared of adipose tissues, ovary, and other connective tissues and cut into a strip of about 1 cm in length. A myometrial strip was then placed in a 20 mL-double jacket organ chamber pre-filled with normal physiological salt solution, Jalon-Ringer solution, containing (in mM) 154.0 NaCl, 5.63 KCl, 0.648 CaCl₂, 5.95 NaHCO₃ and 2.77 Glucose. In some experiments, other physiological solutions were required as indicated in experimental procedures. Their compositions were as follows: 1) Lock-Ringer solution (in mM) 154.0 NaCl, 5.63 KCl, 2.16 CaCl₂, 5.95 NaHCO₃, 2.10 MgCl₂ and 5.55 Glucose 2) Depolarizing solution (in mM) 103.3 NaCl, 56.3 KCl, 0.648 CaCl₂, 5.95 NaHCO₃ and 2.77 Glucose and 3) Ca²⁺-free solution (similar to depolarizing solution but lacking CaCl₂) (in mM) 103.3 NaCl, 56.3 KCl, 5.95 NaHCO₃, 2.77 Glucose and 0.01 EDTA. These solutions were saturated with carbogen (95 % O₂, 5 % CO₂) and the temperature was kept constantly at 37 °C throughout the experiment. The myometrial strip was then fixed in position in the organ chamber and connected to force displacement transducer (Grass Instrument Co.) to record the contractile responses isometrically on the adjoining Grass polygraph. The myometrial strip was equilibrated at initial tension of 2 g for at least 1 h during which the medium was changed every 15 min. The medium was changed to fresh medium before the experiment was begun. In all experiments, a myometrial strip from the other uterine horn of the same animal was also set up in parallel to serve as a control treatment to compare with the drug effect. This myometrial strip was prepared with similar procedures to the drug treated strip, but the relevant vehicle of the drug being tested was used to substitute the drug solution. In all experiments which multiple concentrations of drug were tested in the same myometrial strip, an appropriate amount of drug was added to the organ chamber cumulatively to achieve each concentration as required.

Experimental procedures

Effect of papaya alkaloid (PA) on myometrial contraction

After the myometrial strip was equilibrated for 1 h, PA (10⁻⁶ - 10⁻³ gm/mL) were added cumulatively to the organ chamber. Changes in myometrial tension were then recorded.

Effect of PA on myometrium pre-contracted with depolarizing solution

After the myometrial strip was equilibrated for 1 h, Jalon-Ringer solution in the organ chamber was changed to a depolarizing solution to induce contraction of the myometrial strip [11]. When the contraction reached the maximum plateau, PA (10⁻⁶ - 10⁻³ gm/mL) were added to the organ chamber. The contractile response caused by each concentration of PA was permitted to develop maximally before adding the next concentration of PA. The control myometrial strip was tested in parallel by the addition of PA vehicle. Changes in myometrial tension were then recorded.

Effect of PA and verapamil on myometrial contraction produced by CaCl₂ in Ca²⁺-free solution

After the myometrial strip was equilibrated for 1 h, the medium was replaced by Ca²⁺-free solution [11]. This solution was replaced by fresh solution every 10 min until the tension returned to baseline. CaCl₂ (10⁻⁵ - 3×10⁻² M) were then added to the organ chamber. When the maximum contraction was achieved, the myometrial strip was then washed with Ca²⁺-free solution for many times until the myometrial tension return to baseline. The myometrial strip was then pre-incubated in PA (10⁻⁵ gm/mL) or verapamil (10⁻⁹ M) for 15 min and cumulative concentrations of CaCl₂ (10⁻⁵ - 3×10⁻² M) were added. The contractile response to each concentration of CaCl₂ were recorded. This experiment was repeated but the concentration of PA was changed to 3×10⁻⁵ and 10⁻⁴ M or verapamil at 10⁻⁸ and 10⁻⁷ M.

Effect of PA on myometrial contractility induced by uterine stimulants

The myometrial strip was incubated in Lock-Ringer solution for 1 h at 37 °C. Myometrial contraction was induced by the addition of either oxytocin (1 mU/mL), acetylcholine (3×10⁻⁵ M) or PGF_{2α} (10⁻⁵ M). After the contractile responses to the stimulants were stable, cumulative concentrations of PA (10⁻⁶ - 10⁻³ gm/mL) were added to the organ chamber. The contractile responses of myometrium to each concentration of PA were permitted to develop maximally before the addition of the next concentration. The control myometrial strip was performed in parallel. Changes in myometrial tension were then recorded.

Effect of β -adrenergic receptor antagonist on myometrial relaxation produced by PA or isoproterenol

After equilibration for 1 h, the myometrial strip was induced to contract by exchanging the solution in the organ chamber to a depolarizing solution. After tonic contraction plateau was achieved, cumulative concentrations of PA (10^{-6} - 10^{-3} gm/mL) or isoproterenol (10^{-10} - 10^{-5} M) were added to the organ chamber and the contractile responses of the myometrial strip were recorded. The myometrial strip was then washed for many times with Jalon-Ringer solution. The procedures were then repeated but the myometrial strip was incubated for 20 min. with propranolol (10^{-7} M), a non-selective β -adrenergic receptor antagonist, before the addition of isoproterenol or PA. The control strip was performed in parallel. The myometrial tension caused by PA or isoproterenol with or without propranolol was then recorded.

Data analysis

The responses of the myometrium were analyzed as changes in isometric tension and calculated as percentage of maximum contraction induced by the uterine stimulants. The results were expressed in term of mean \pm standard error of mean (mean \pm S.E.M.). The IC_{50} (half maximal inhibitory concentration) was calculated from a plot of log concentration vs. the percentage of maximum contraction response in individual experiment. Repeated measure ANOVA and Duncan's multiple range test were used for statistical analysis. A significant difference will be determined when p is less than 0.05.

Results and discussion

Effect of papaya alkaloid (PA) on myometrial contraction

Cumulative concentrations of PA (10^{-6} - 10^{-3} gm/mL) had no effects on the myometrial strip as observed by no changes in resting tension of the myometrial strip to each concentration of PA (data not shown).

Effect of PA on myometrium pre-contracted with depolarizing solution

In the control treatment, the vehicle of PA had no effects on the contractile response of the myometrial strip produced by depolarizing solution. On the contrary, PA (10^{-6} - 10^{-3} gm/mL) inhibited the contraction of myometrial strip in a concentration-dependent fashion. The inhibition occurred significantly with PA at the concentration of 10^{-5} - 10^{-3} gm/mL while PA at the concentration of 10^{-3} gm/ml caused the myometrial strip to relax completely. The IC_{50} of PA was $8.37 \pm 0.58 \times 10^{-5}$ gm/mL. The concentration-response curve of PA on myometrial contraction induced by depolarizing solution was shown in **Figure 1**.

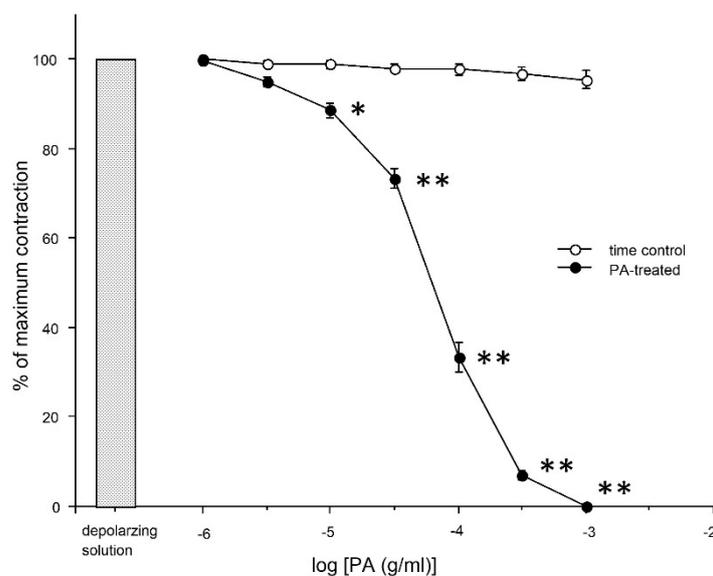


Figure 1 The concentration-response relationship of PA in myometrial strip with depolarizing solution-induced contraction. Each data point expressed as the mean \pm S.E. of 8 experiments. (* = $p < 0.05$, ** = $p < 0.01$ as compared to the control).

Effect of PA and verapamil on myometrial contraction produced by CaCl₂ in Ca²⁺-free solution

In Ca²⁺-free solution, the addition of CaCl₂ (10^{-5} - 3×10^{-2} M) caused the myometrium to contract in a concentration-dependent fashion. The presence of PA (10^{-5} , 3×10^{-5} and 10^{-4} gm/mL) inhibited the contraction significantly ($p < 0.05$ for 10^{-5} gm/mL, $p < 0.01$ for 3×10^{-5} gm/mL and $p < 0.01$ for 10^{-4} gm/mL). The concentration-response curves of CaCl₂ were shifted rightward and the maximum contraction was decreased. EC₅₀ of CaCl₂ alone was $4.74 \pm 0.68 \times 10^{-4}$ M, but the value was changed to $1.38 \pm 0.17 \times 10^{-3}$ ($p < 0.05$), $2.60 \pm 0.26 \times 10^{-3}$ ($p < 0.01$) and $7.50 \pm 0.44 \times 10^{-3}$ M ($p < 0.01$) in response to the presence of PA at 10^{-5} , 3×10^{-5} or 10^{-4} gm/mL, respectively. Likewise, the myometrial contraction induced by CaCl₂ was also suppressed by verapamil in a concentration-dependent fashion. In the presence of verapamil (10^{-9} , 10^{-8} and 10^{-7} M), the myometrial contraction produced by CaCl₂ were inhibited significantly ($p < 0.05$ for 10^{-9} M, $p < 0.05$ for 10^{-8} M and $p < 0.05$ for 10^{-7} M, as compared to the tension in the absence of verapamil). The concentration-response curves produced by CaCl₂ (10^{-5} - 3×10^{-2} M) were shifted rightward whilst the maximum contraction was also decreased in comparison to the effect of only CaCl₂. EC₅₀ of CaCl₂ alone was $5.59 \pm 1.78 \times 10^{-4}$ M, but the value was elevated significantly to $1.20 \pm 0.26 \times 10^{-3}$ M ($p < 0.05$), $2.51 \pm 0.11 \times 10^{-3}$ M ($p < 0.01$), and $5.83 \pm 0.15 \times 10^{-3}$ M ($p < 0.01$) in the presence of verapamil 10^{-9} , 10^{-8} or 10^{-7} M, respectively. The log concentration-response relationship of CaCl₂ with or without PA or verapamil at various concentrations were illustrated in Figure 2.

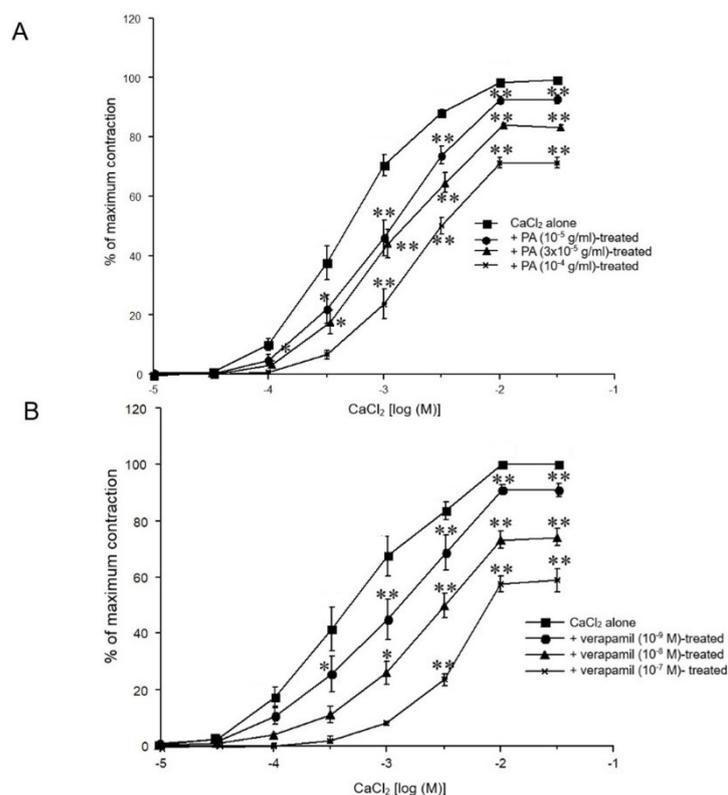


Figure 2 The concentration-response relationship of CaCl₂ (10^{-5} - 3×10^{-2} M)-induced contraction in myometrium with or without A) PA, or B) verapamil. The data were expressed in term of mean \pm S.E. of 8 experiments. (* = $p < 0.05$, ** = $p < 0.01$ when compare to the effect of CaCl₂ alone).

Effect of PA on myometrial contraction induced by oxytocin, PGF_{2 α} or acetylcholine

In the control group, oxytocin (1 mU/mL), PGF_{2 α} (10^{-5} M) or acetylcholine (3×10^{-5} M) induced a phasic contraction of the uterus of which the amplitude was steady throughout the experiment. The vehicle of PA had no effects on the contractile responses produced by these stimulants. On the contrary, PA (10^{-6} - 10^{-3} gm/mL) significantly inhibited the amplitude of contraction induced by either oxytocin,

PGF_{2α} or acetylcholine. The concentration-response relationship of PA on the myometrial contractility produced by oxytocin, PGF_{2α} or acetylcholine were illustrated in **Figure 3**, IC₅₀ values of PA on the contraction of myometrial strip pre-contracted with oxytocin, PGF_{2α} or acetylcholine were $7.39 \pm 1.35 \times 10^{-5}$, $8.27 \pm 1.80 \times 10^{-5}$ or $6.07 \pm 1.43 \times 10^{-5}$ gm/mL, respectively.

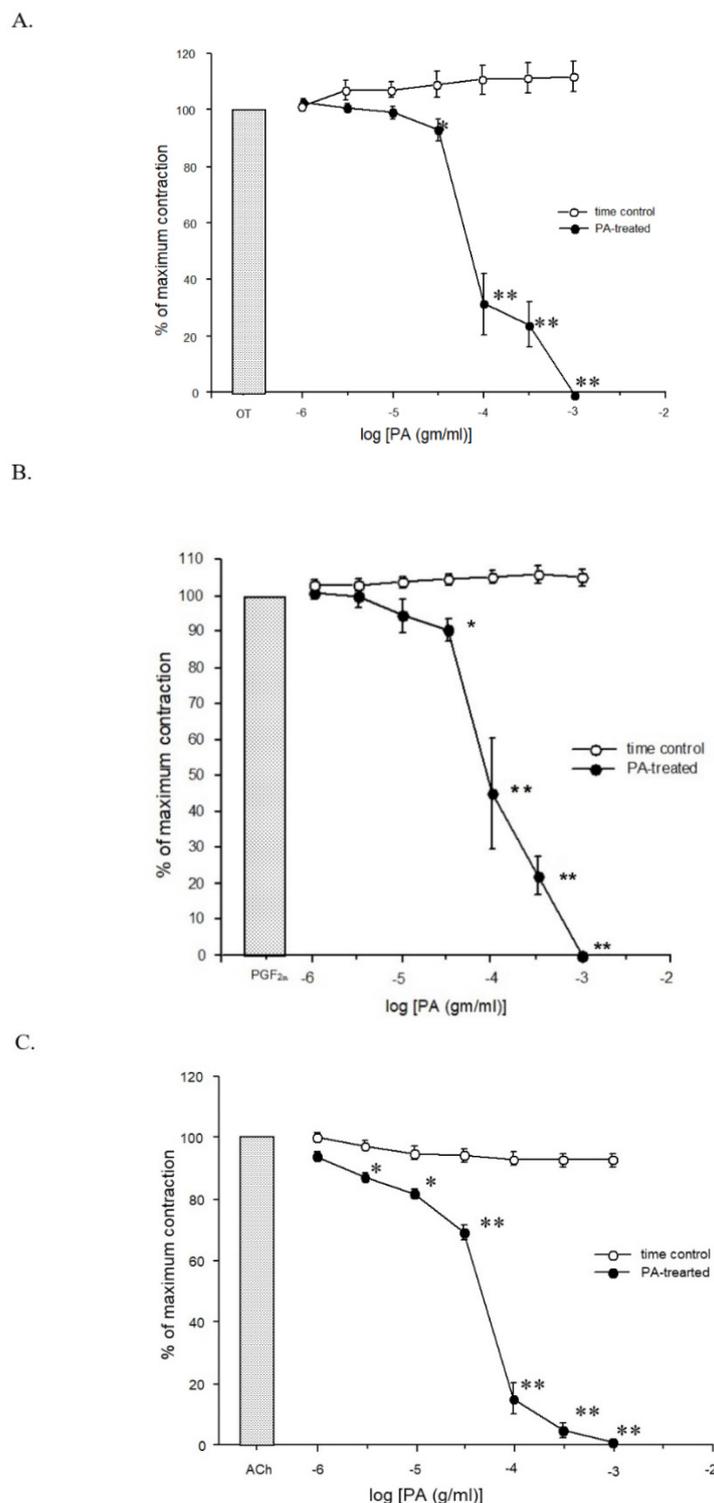


Figure 3 The concentration-response relationship of PA on myometrium contraction produced by A) oxytocin (1 mU/mL), B) PGF_{2α} (10^{-5} M) or C) acetylcholine (3×10^{-5} M). The data was expressed in term of mean \pm S.E. of 8 experiments (* $p < 0.05$, ** $p < 0.01$ in comparison to the respective control).

Effect of propranolol on myometrial relaxation induced by PA or isoproterenol

This experiment was performed initially by inducing the uterus to contract by depolarizing solution. This tonic contraction when the maximum plateau was achieved was stable throughout the experimental period. The addition of vehicle of PA or isoproterenol produced no effects on this contraction. The addition of PA (10^{-6} - 10^{-3} gm/mL) inhibited the contraction with IC_{50} of $6.28 \pm 0.62 \times 10^{-4}$ gm/mL. In the presence of propranolol (10^{-7} M), PA also suppressed the contraction with IC_{50} of $6.35 \pm 0.32 \times 10^{-4}$ gm/mL which was not significant difference from the effect in the absence of propranolol ($p > 0.05$). The addition of isoproterenol (10^{-10} - 10^{-5} M) appeared to inhibit the myometrial contractility in a concentration-dependent fashion with IC_{50} of $1.39 \pm 0.23 \times 10^{-9}$ M. The presence of propranolol (10^{-7} M) diminished the activity of isoproterenol significantly ($p < 0.01$) as noted by the rightward shift of concentration-response curve of isoproterenol with IC_{50} of $1.72 \pm 0.38 \times 10^{-6}$ M. The plot of concentration-response relationship of PA or isoproterenol in the absence or presence of propranolol (10^{-7} M) were illustrated in **Figure 4**.

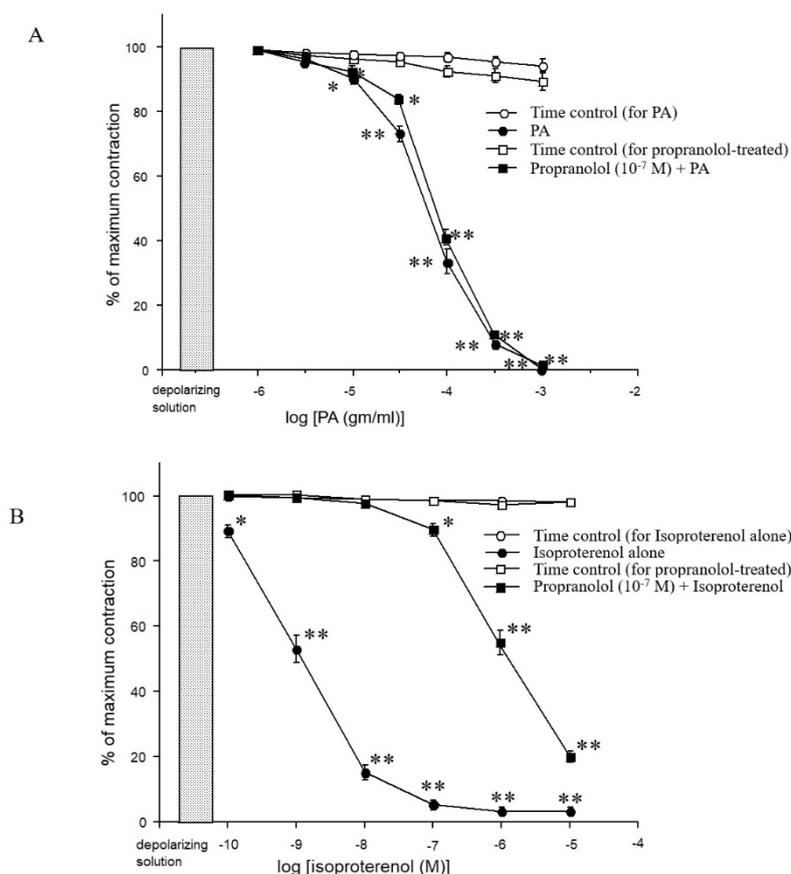


Figure 4 The concentration-response relationship of PA A) or isoproterenol B) and myometrial relaxation in the absence or presence of propranolol (10^{-7} M) in depolarizing solution-contracted myometrium. The data were expressed in terms of mean \pm S.E. of 8 experiments. (* $p < 0.05$, ** $p < 0.01$ in comparison to the control).

Discussion

The aim of this study is to demonstrate the action and possible mechanism of papaya alkaloid (PA) on the rat myometrium. The crude alkaloid extract (9.1 gm) was prepared by a single extraction and used throughout this study. The yield of crude alkaloid extract was approximately 0.414 % of the dried leaves weight. Carpaine was reported to be a principal alkaloid of *Carica papaya* L. which was found to be present 0.52 % in shade-dried leaves [12]. Apart from carpaine, pseudocarpaine, dehydrocarpaine I and II have been found in papaya leaves [2,3], however, the yield of these alkaloids has not been published.

Our preliminary finding showed that PA had no effect on myometrial contraction. However, PA was able to completely inhibit myometrial contraction produced by depolarizing solution and caused a rightward shift of concentration-response curve of CaCl_2 . It is suggested that the effect of PA may involve a decrease in Ca^{2+} entry into the myometrial muscle cells. Furthermore, the relaxing activity of PA on CaCl_2 -induced myometrial contraction in Ca^{2+} -free solution is resemble to the effect of a calcium channel antagonist, verapamil [13]. It is suggested that the inhibitory effect of PA on Ca^{2+} entry through voltage-dependent Ca^{2+} -channel may occur in the same fashion as verapamil.

It is well established that the uterus contains various specific receptors for many classes of myometrial stimulants and myometrial relaxants. Oxytocin, $\text{PGF}_{2\alpha}$ and ACh are known to be natural-occurring myometrial stimulants. The interaction of these stimulants on their own receptors on uterus (OXTR for oxytocin, FP receptor for $\text{PGF}_{2\alpha}$, and M2 and M3 receptor for ACh) can induce myometrial contraction [14]. At present, there are no known agonists or antagonists that can cross-react with these 3 types of receptors. PA appeared to be a general uterine relaxant which was able to inhibit myometrial contraction caused by those stimulants as well as depolarizing solution and CaCl_2 . It seems unlikely that PA could produce the effect by interfering the binding of those stimulants to their receptors.

Our results have shown that PA inhibited K^+ -induced uterine contraction using depolarizing solution. It also inhibited the contraction induced by cumulative concentrations of CaCl_2 in Ca^{2+} -free solution (K^+ -depolarized uterus) similar to that occur by verapamil. These results suggested that the uterine relaxing effect of PA may involve mainly Ca^{2+} influx, probably via voltage-operated calcium channels.

The action of oxytocin, $\text{PGF}_{2\alpha}$ and ACh which induce the myometrium to contract is the result of an increase in intracellular Ca^{2+} from both Ca^{2+} -release from internal storage and probably Ca^{2+} -entry via plasma membrane [14-16]. The action of oxytocin, $\text{PGF}_{2\alpha}$ and ACh which induced Ca^{2+} -release from internal storage are postulated to be mediated by IP_3 signaling pathway. It is well known that all receptors of oxytocin, $\text{PGF}_{2\alpha}$ and ACh are G-protein-coupled receptors (G_q subtype) [17,18]. The interaction between these agonists to their receptors on plasma membrane activates G-protein coupled to phospholipase C that causes the hydrolysis of PIP_2 to IP_3 . IP_3 will then activate its receptor on sarcoplasmic reticulum resulting in Ca^{2+} -release from internal storage. The evidence has shown that the effect of oxytocin is mediated through the hydrolysis of PIP_2 to IP_3 to induce Ca^{2+} release. Nevertheless, the contractile effect of oxytocin is noticeably suppressed by verapamil, a Ca^{2+} channel blocker [19]. It is indicated that an increase in Ca^{2+} entry from extracellular space through L-type voltage-dependent Ca^{2+} channel mediated mainly the action of oxytocin to cause myometrium contraction. A recent study suggested that oxytocin open the voltage-operated calcium channels indirectly via the opening of other cation channels such as Ca^{2+} -activated Cl^- channels or capacitative Ca^{2+} entry which cause depolarization and open the calcium channel [16]. In addition, oxytocin-induced Ca^{2+} influx have been shown to be involved several Ca^{2+} channels in human myometrial cells such as transient receptor potential cation channels (TrpC)1, TrpC3, TrpC4, and TrpC6 [20]. The present of these channels in rat myometrial cells has yet to be determined. If they are present, these channels may contribute to a rise in intracellular Ca^{2+} in rat myometrial cells and might be the target to cause the effect of PA. Although, the involvement of $\text{PGF}_{2\alpha}$ and ACh to stimulate the Ca^{2+} entry via the opening of voltage-operated calcium channels is unclear, Ca^{2+} entry via these channels is essential for myometrium contraction [15].

The β -adrenergic receptors in the myometrium are mainly of β_2 -subtype and the activation of β_2 receptors cause myometrial relaxation [21]. The present study has shown that nonspecific β -adrenergic receptor agonist, isoproterenol (10^{-10} - 10^{-5} M), caused relaxation of KCl-depolarized myometrium in a concentration-dependent fashion, and this effect was abolished by pretreatment with a β -adrenergic receptor antagonist propranolol (10^{-7} M). PA alone caused a relaxation of the KCl-depolarized uterus similar to the effect of isoproterenol. Although propranolol (10^{-7} M) was able to inhibit the effect of isoproterenol, it produced no effect on the relaxation activity caused by PA. This result suggested that the myometrial relaxation caused by PA may be unrelated to the interaction of PA on β_2 -adrenergic receptor.

β_2 -adrenergic receptor is known to be a G-protein coupled receptor of the G_s subtype. The interaction of β -adrenergic agonist on this receptor causes the stimulation of adenylate cyclase which accelerates the conversion of ATP to cAMP. The rise in intracellular cAMP then activates cAMP-dependent kinase which phosphorylates proteins and causes myometrial relaxation [14]. The termination of the effect occurred by the hydrolysis of cAMP. This reaction is catalyzed by several phosphodiesterase (PDE) which convert cAMP to 5'AMP [22]. The inhibition of this enzyme by PDE inhibitor may cause a rise in intracellular cAMP leading to myometrial relaxation [23]. Although the effect of PA is unlikely to be mediated via β_2 -adrenergic receptor activation, a PDE inhibition may be the site of action of PA. In

addition, other intracellular sites such as the contractile machinery of which its activity is downstream after the interaction of Ca^{2+} to calmodulin may be the site of action of PA to cause uterine muscle relaxation.

Conclusions

The results of this study indicate that PA is a myometrial relaxant. The myometrial relaxation effect of PA is unlikely to be mediated by an activation of β_2 -adrenergic receptor. The effect of PA may involve an inhibition of a rise in intracellular Ca^{2+} from both Ca^{2+} release from internal storage and Ca^{2+} entry via plasma membrane. This action may be mediated predominantly by a reduction on Ca^{2+} influx, possibly by an inhibition on voltage-operated Ca^{2+} channel. However, the precise mechanism for the relaxing effect of PA is awaited to be determined.

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