



Effects of Crude Alcoholic Extract of *Piper chaba* Vahl.(Deeplee) on Contractility of Isolated Rat Vas Deferens

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Introduction

Piper chaba (syn. *P. retrofractum* Vahl, Piperaceae) is widely distributed in Southeast Asia. The fruit of this plant is commonly called "Deeplee" in Thailand and has been used as an anti-flatulent, expectorant, antitussive, antifungal, uterine-contracting agent, sedative-hypnotics, appetizer, and counter-irritant in the traditional medicine of Thailand. The main constituents of Deeplee are piperine, chavicine and volatile oil.¹ These active compounds from the fruit of Deeplee also have been used for improving food digestion, blood circulation, asthma, influenza, hypertension and overall health. They also had antifatulent and antidiarrhoeal activities in mice.² The extract of Deeplee has been known to reduce KCl – induced contraction of pig aorta and increased KCl–induced contraction of pig kidney.³ In addition, crude alcoholic extract of Deeplee caused relaxation of vas deferens pretreated with norepinephrine (NE), KCl, BaCl₂ and serotonin (5-HT). The extract, however, could induce vas deferens contractility in normal condition.⁴ The aim of this study, therefore, was to investigate the effect of crude alcoholic extract of Deeplee on smooth muscle of vas deferens of the Wistar rats.

Methods

Plant material

Fruits of *Piper chaba* Vahl.(500 g) were collected from Thailand. These fruits were washed, cut into pieces, crushed into powder and then extracted by maceration with 95 % ethanol for 3 days. The clear solution was collected and the remaining powder was reextracted with 95 % ethanol. The total extract was filtered and then evaporated. 1 g of this crude extract was added with 10 mL of 95% ethanol and used as stock solution.

Chemicals

Norepinephrine (NE) and prazosin were obtained from Sigma. All other chemicals used for various assays were of analytical grade and were obtained from local commercial sources.

Animal and isolated organ preparations

Male Wistar rats weighing 250 – 350 g were sacrificed by stunning followed by cervical dislocation. The rats were maintained in rat cages under standard conditions of light and dark (12 h: 12 h). They were fed with standard laboratory chow and tap water ad libitum. Research protocols and care of rats were based on the principles and guidelines adopted by the guide for the care and use of laboratory animals (Thai NIH revised in 2011) and approved by the local ethical committee of Huachiew Chalermprakiet University, Thailand.

The vas deferens was quickly dissected out and placed in a petridish containing Krebs-Henseleit solution (119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25 mM NaHCO₃ and 5.6 mM glucose). The vas deferens was cleared of connective tissue, fat, and cut into rings 2 - 2.5 mm in length of prostatic and epididymal ends. Each vas deferens ring was fixed between two holders inside a 15 mL capacity organ bath containing the bathing Krebs's solution (37°C, gassed with 95% oxygen and 5% carbon dioxide). While one holder was fixed to the organ bath, the other to a 5 g isometric transducer (Heto Lab Equipment BIOPAC System, Scientific Promotion Co. Ltd., Model MP 100 1759) which connected through data acquisition equipment to a computer.

Experimental Design

A basal tension of 1 g was applied to the vas deferens rings. After a 90 minute period of incubation, NE at a concentration of 10^{-5} M was added to the bathing solution in the organ bath. An initial rise in tension occurred, followed by a plateau which was maintained for 30 minutes. Contraction was recorded by means of tension. Effect of 95% ethanol on the contraction of vas deferens was also studied.

Effect of crude alcoholic extract of Deeplee on vas deferens contraction

For the tests, the same amount of cumulative dose of 15, 150 and 300 mg/mL of crude alcoholic extract of Deeplee was added to separate baths. The bathing solution was replaced by fresh solution every 20 minutes in the organ baths. After each experiment the tissues were washed 5 times with the bathing solution.

Effect of prazosin on vas deferens contraction induced by 150 mg/ml of crude alcoholic extract of Deeplee

After the tension of vas deferens ring was kept stable, 150 mg/mL of crude alcoholic extract of Deeplee was added to the baths. The tension was recorded for 15 minutes prior to addition of 10^{-5} M prazosin. The tension was recorded for 5 minutes after prazosin exposure. Then, 10^{-5} M NE was added to induce vas deferens ring contraction. The tension was recorded for 5 minutes prior to addition of 150 mg/ml of crude alcoholic extract of Deeplee again.

Statistical Analysis: Analyses were performed with computer. Results are reported as the mean of percent contraction \pm standard error of the mean. The two-sample one-way analysis of variance (ANOVA) was used to detect differences in the means between groups, using the Student-Newman-Keuls test ($p < 0.05$)

Results

Effect of crude alcoholic extract of Deeplee on vas deferens contraction

In the isolated organ bath assay, epididymal or prostatic ends of rat vas deferens were stimulated with 10^{-5} M NE. The contraction profiles of both epididymal and prostatic ends in response to 10^{-5} M NE were similar, consisting of phasic and rhythmic contractions. After this stimulation, the epididymal end produced an initial fast phasic component followed by a tonic response, while the prostatic end produced a fast phasic response without tonic component. The mean of tension on phasic contraction induced by 10^{-5} M NE was 0.96 ± 0.18 g ($n=6$) for epididymal end and 0.33 ± 0.11 g ($n = 6$) for prostatic end. This NE-induced contraction of the vas deferens was inhibited by 10^{-5} M prazosin.

In cumulative dose, the crude alcoholic extract of Deeplee produced a concentration-dependent contraction of the vas deferens. 150 mg/mL of Crude alcoholic extract of Deeplee induced rhythmic contraction of both epididymal end (0.21 ± 0.04 g, $n = 6$) and prostatic end (0.20 ± 0.06 g, $n = 4$). One dose of 150 mg/mL of crude alcoholic extract of Deeplee induced rhythmic contraction on epididymal end (0.1802 ± 0.04 g, $n = 4$). The mean tension in prostatic end were 0.3779 ± 0.05 g ($n = 4$).

The contraction could be observed in the second exposure of 150 mg/mL crude alcoholic extract of Deeplee. The tension increased from the first exposure by 24.38 ± 0.04 % in the epididymal end and 137.27 ± 0.04 % in the prostatic end. (Figure 1)

Effect of prazosin on vas deferens contraction induced by 150 mg/ml of crude alcoholic extract of Deeplee

The α_1 -adrenoceptor antagonist, prazosin was tested in the experiment. Pre-treatment of the tissues with 10^{-5} M NE for 20 minutes resulted in the appearance of contractions. These contractions were abolished by 10^{-5} M prazosin. In contrast, the same amount of prazosin was not able to completely inhibit rhythmic contraction induced by 150 mg/mL of the crude alcoholic extract of Deeplee. Rhythmic contraction of the epididymal end induced by 150 mg/mL of crude alcoholic extract of Deeplee was significantly decreased by 31.45 ± 6.21 % ($n = 5$) after adding prazosin. Nonsignificant decrease in the contraction by 28.40 ± 10.76 % ($n=4$) was found in case of prostatic end.(Figure 2) Repeat addition of 10^{-5} M NE after prazosin caused rhythmic but not phasic contractions in both epididymal and prostatic ends.

After repeat addition of crude alcoholic extract of Deeplee, the rhythmic contraction of prostatic end increased more than first exposure. The tension induced by crude alcoholic extract of Deeplee were 147.66 ± 47.68 % ($n = 4$) in prostatic end and 77.28 ± 34.55 % ($n = 5$) in epididymal end. (Figure 2, 3)

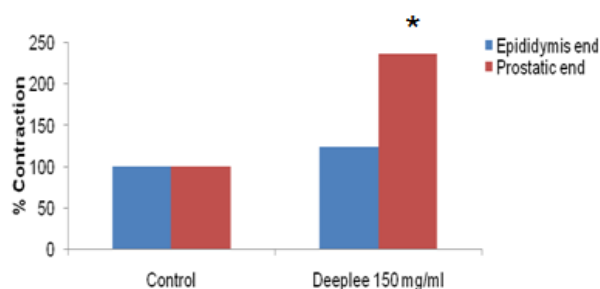


Figure 1 Effect of repeat 150 mg/mL of the crude alcoholic extract of Deeplee on contraction of epididymal end (blue bar) and prostatic end (red bar) of rat vas deferens. All plots are mean \pm SEM of (n = 6). Control bars is first exposure the crude alcoholic extract of Deeplee.

*represents statistical significance

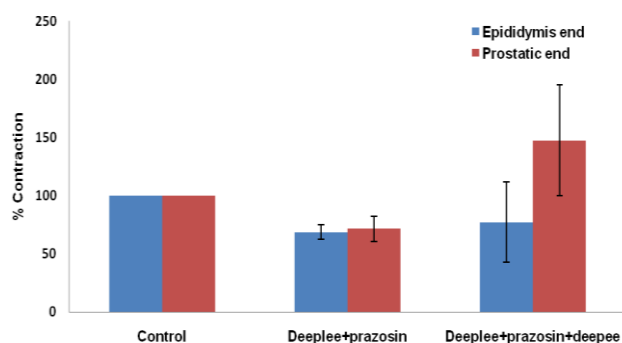


Figure 2 Effect of 10^{-5} M prazosin on vas deferens contraction induced by 150 mg/mL of the crude alcoholic extract of Deeplee. All plots are mean \pm SEM of (n = 6). Control bars is first exposure the crude alcoholic extract of Deeplee.

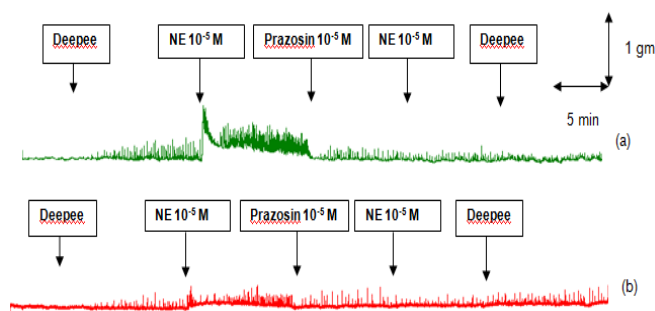


Figure 3 Representative tracing shows the effect of 10^{-5} M prazosin on contraction of rat vas deferens induced by 150 mg/mL of crude alcoholic extract of Deeplee (a) epididymal end (b) prostatic end

Discussion

The results of this study show that 150 mg/mL of the crude alcoholic extract of Deeplee could induce rhythmic contraction of both epididymal and prostatic ends of the rat vas deferens. After the test compound was washed out and the isolated vas deferens was incubated until stabilization, crude alcoholic extract of Deeplee were regiven. This second exposure caused higher contraction.

The result of this study demonstrated that crude alcoholic extract of Deeplee had an activity to induce vas deferens contractility. They might be able to increase extracellular Ca^{2+} entry through L-type Ca^{2+} channels which was essential for muscle activity. Kawada T, Sakabe S, Watanabe T, Yamamoto M and Iwai K also concluded in one study that there was a release of endogenous catecholamine which in turn induced a slowly developing contracture in the rat vas deferens. Release of catecholamines by piperine, one of the main constituents of Deeplee, also has been demonstrated in various tissues.⁵ Kulshrestha VK, Singh N and Srivastava RK also reported about the endogenously released catecholamines induced by piperine in thoracic aorta and vessels.⁶ As known, catecholamine action is often mediated through receptor-operated calcium channel.⁷ However, our result was not enough to confirm this suggestion. Interestingly, reexposure of crude alcoholic extract of Deeplee in our study still cause contraction higher than the first one which should not be found in case of indirect effect (Figure 1). Direct effect of crude alcoholic extract of Deeplee on alpha receptors was examined in our study. As known, NE is the α_1 -adrenoceptor agonist, acting via inositol trisphosphate leading to increase in intracellular Ca^{2+} and the slow component of nerve-mediated contraction. In the NE stimulation, the rat epididymal and prostatic end response were initially phasic and then followed by a tonic component.^{8,9,10} One study demonstrated α_1 -adrenoceptor antagonist activity in vas deferens. In both epididymal and prostatic ends, doxazosin showed the strongest α_1 -adrenoceptor antagonist activity while terazosin displayed a weak antagonist effect.¹¹ 10^{-5} M prazosin in our study was able to inhibit rhythmic contraction of vas deferens induced by 150 mg/mL of crude alcoholic extract of Deeplee. This inhibition,

however, was not complete because we still found some rhythmic contraction. Reexposure to 150 mg/mL of crude alcoholic extract of Deeplee could increase rhythmic contraction, therefore, the effect of crude alcoholic extract of Deeplee on vas deferens might be mediated through other mechanism not related to alpha receptor.

Increase contraction of vas deferens has been known to increase sperm movement.¹² Therefore, crude alcoholic extract of Deeplee might have some benefit for ejaculatory disorders. Previous study also showed that 100 mg/day dosage of crude extract of Deeplee could increase blood testosterone level in 7 from 9 males with hypogonadism and could have some weak androgenic activity. It also could increase the frequency of coitus in male hypogonadism.¹³ Many drugs including α 1-adrenoceptor antagonists which were used for treatment of hypertension or benign prostatic hyperplasia have been known to cause ejaculatory disorders. α 1-Adrenoceptor antagonists also have been reported to decrease secretion of sperm, have some effects on movement and storage of sperm in testis and epididymis¹⁴. Thus, the potential effect of crude alcoholic extract of Deeplee on vas deferens shown in our study might also counteract the sexual side effect of these drugs.

Conclusion

In conclusion, crude alcoholic extract of Deeplee in the doses used in our study could enhance rhythmic contraction of rat vas deferens which mechanism not related to alpha receptors. Whether this effect could have benefit for ejaculatory disorders need further studies.

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