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Effects of *Butea superba* (Roxb.) on penile erection in diabetic rats

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Abstract

We investigate the effect of ethanolic extract of *Butea superba* (Roxb.) on erectile dysfunction in diabetic rats by measurement of intracavernous pressure (ICP) and on cavernosal smooth muscle relaxation. Male Sprague-Dawley rats were induced to become diabetic by a single intravenous injection of Streptozotocin; STZ (55 mg/kg body weight; BW). The ethanolic extract at the concentration of 1, 10 and 100 mg/kg BW were administered orally once a day to diabetic rats in each group for 4 weeks. Diabetic rats showed a significant decreased both ICP and the relaxation of the cavernosal smooth muscle compared to the normal rats. The extract of *B. superba* significantly increased the ICP with the effective dose of 10 mg/kg BW (61.00 ±11.11 mmHg vs 39.61± 11.01 mmHg in diabetic control group). Moreover, *B. superba* treated group also enhanced the relaxation of cavernosal smooth muscle with the EC_{50} 1.17 mg/ml. These results suggest that the extract of *B. superba* enhanced penile erection in diabetic rats by increasing the ICP that might be explained by increasing in blood flow as a result of relaxing cavernous smooth muscle.

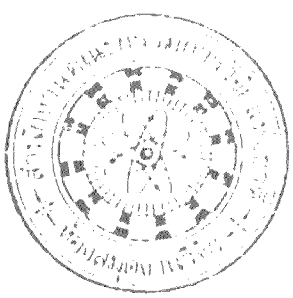
Keywords: *Butea superba* Roxb., diabetic mellitus, erectile dysfunction, intracavernous pressure

1 Introduction

Erectile dysfunction (ED) is very common complication among diabetic patients. Diabetic men suffered from ED at a much higher incidence than the normal men. About 75% of diabetic men with young age will develop to ED. The impairement of both neutral and vascular pathways are necessary for penile erection, wre found in diabetic men. Several studies demonstrated that treatment diabetic ED with phophodiesterase5 inhibitors were attenuated the development of the ED and also improved the endothelial function.

Butea superba (Roxb.), known as "Kwaao Khruea Daeng" in Thai, is a plant in the *Papilionaceae* family [3, 4]. It is almost found in the northern region of Thailand. *B. superba* has been used as a natural product for physical and mental strength and for prevention of age-related health problems. In Thai traditional medicine, *B. superba* is a rejuvenating herb for men [5]. Compounds extracted from *B. superba* are effective in inhibiting cAMP phosphodiesterase *in vitro*, the mechanism of which has been shown to play an important role in penile erection [6]. Previous studied, it was shown that *B. superba* induces a penile erection by increasing ICP with the maximum effective dose at 1 mg/kg BW and relaxed the corpus cavernosal smooth muscle in rat models [7]. Thai clinical trial of *B. superba* powder treatment in the Thai male volunteers showed effective treatment of ED [8].

Although it has been reported that *B. superba* can induce a penile erection, its efficacy for treatment of erectile dysfunction in diabetes is still no explored. Therefore, the aims of this experiment were to determine whether *B. superba* alcoholic extract can reverse penile erection by increasing ICP and relaxing cavernous smooth muscle in streptozotocin-induced diabetic rats, which ultimately results in improvement of erection function.



2 Materials and methods

2.1 Chemicals and reagents

Streptozotocin, phenylephrine, isobutylmethylxanthine (IBMX) and other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Preparation of plant extract

Tuberous roots of *B. superba* were collected from Phrae provinces in November, 2006, Thailand and identified by Associate Professor Yuthana Smitasiri. This plant was re-identified by the Botany and Weedscience Division, Department of Agriculture, Kasetsart University, Bangkok, with the sample voucher code of MFLU 310. The roots were slices into small pieces and extracted with ethanol. The ethanolic extract was evaporated under reduced pressure at 45°C using a rotary evaporator. The extracts were stored at -20°C until used.

2.3 Animals

Male sprague-Dawley rats (age 8 weeks) were obtained from the National Laboratory Animal Center, Mahidol University, Nakornpathom, Thailand. The animals were housed under a 12:12-hour light-dark cycle and maintained at 24±1°C. Animal feed and water was supplied *ad libitum*. The protocol had been approved by the Naresuan University Ethical Committee.

2.4 Treatment

Rats were randomized into five groups (10 rats/ group). Diabetes was induced in animals by a single intravenous injection of Streptozotocin (STZ; 55 mg/kg BW). The control group was intravenously injected with citrate buffer. The blood glucose levels were monitored using a glucose test kit (Accu-check advantage II glucose test kit,

Roche diagnostic Ltd., Switzerland) each week after induction. Animals with a blood glucose level of 300 mg/dl or higher were chosen for the further study. The diabetic rats were divided into four groups and treated with distilled water (or diabetic control; DM), 1, 10 and 100 mg/kg BW/day of *B. superba* alcoholic extract orally for four weeks. After that, they were used for the ICP, mean systemic arterial pressure (MAP) and cavernosal smooth muscle relaxation.

2.5 Penile erection in diabetic rats

The erectile erection was assessed by monitoring the ICP following electrostimulation of cavernous nerves [ ]. Rats were anesthesized by intraperitoneal injection of pentobarbitone sodium. The penile skin was incised and the prepuce was degloved to completely expose the corpora cavernosa. A 23 gauge needle connected to polyethylene tubing (Clay-Adams PE-50) filled with physiological saline with 100 IU of heparin/ml was inserted into the corpus cavernosum on one side to measure ICP. A femoral artery was cannulated in a similar fashion with polyethylene tubing to permit continuous monitoring of MAP and connected to a pressure monitor. Both pressure tubes were connected to blood pressure transducers, which were, in turn, connected via transducer amplifiers to a data acquisttion board (MacLab/8e, ADI Instrucments, MA). Each animal received stimulation three times. The animals were allowed to rest until the ICP decreased to the baseline values before the subsequent stimulations were conducted.

2.6 Cavernosal smooth muscle relaxation

The strips of cavernosal smooth muscles were isolated from normal control rats (n=6), diabetic control rats (n=6) and diabetic rats received *B. superba* at the dose of

1, 10, 100 mg/kg BW for 4 weeks. The cavernosal strips were excised and trimmed to the size of about 0.2x0.2x0.5 cm and then, immediately placed in 100% oxygen-saturated HEPES-buffer physiological salt solution (HPSS: 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, 11 mM glucose, pH 7.4). The strip was equilibrated for 2 h with several changes of HPSS. The changes in isometric force were measured using a Mac Lab data acquisition system. The resting tension was pre-contracted with phenylephrine (PE). The relaxation of the cavernosal smooth muscles as a result of *B. superba* extract (0.01-10 mg/ml) was assessed by adding IBMX, a non-selective phosphodiesterase inhibitor, cumulatively to the bathing medium. Data was also performed and presented in term of percentage of relaxation and EC_{50}; the concentration of a compound where 50% of maximal smooth muscle relaxation exhibits a response.

2.7 Statistical Analysis

All data were expressed as mean ± SD. To compare among multiple groups, one way analysis of variance (ANOVA). The least significant difference test (LSD) was employed for comparison between each 2 groups. $P < 0.05$ was considered statistically significant.

3 Results

3.1 General characteristics of diabetic rats

Table 1 showed the general characteristic of the STZ-induced diabetic rats. The diabetic rats demonstrated a significant loss in the total body weight relative to the normal controls. The body weight and blood glucose level of rats treated with *B. superba* was not significantly different from those of diabetic controls.

3.2 Effects of B. superba on MAP

There was no significant change in the MAP upon the treatment with *B. superba* alcoholic extracts in all doses. The MAP of the control group was 96.07±1.43 mmHg. The MAP of the diabetic control animals and treated with the extracts at the concentrations of 1, 10 and 100 mg/kg BW were 96.68±2.12, 94.65±2.10, 95.17±1.29 and 94.06±1.16 mmHg, respectively (Figure 1).

3.3 Effects of B. superba on ICP

The diabetic control rats, the cavernous pressure was 39.61±11.01 mmHg, which was significantly lower than that in normal control rats (65.81±4.82 mmHg). Treatment the animals with extract at the doses of 1, 10 and 100 mg/kg BW significantly induced increases in the ICP compared with the diabetic control group (50.47±12.45, 61.00±11.11 and 51.01±10.00 mmHg, respectively) (Figure 2).

3.4 Effects of B. superba extract on smooth muscle relaxation

After exposing to the various dosages of extract daily for 4 weeks, cavernous smooth muscle was removed from rats and placed in an organ bath. The muscle contraction was induced by exposing to phenylephrine (10^{-6} M). Alcoholic extract of *B. superba* dose-dependently relaxed phenylephrine-induced smooth muscle contraction (Figure 3A). An almost completely relaxation was obtained at the 10 mg/ml of the extract from normal control group. At the highest concentration studied (100 mg/ml) could induce only about 60% relaxation from diabetic control group. The EC_{50} of normal control, diabetic control, and treated group (with extract at the doses

of 1, 10 and 100 mg/kg BW) were about 1.16, 2.32, 1.51, 1.17 and 2.25 mg/ml, respectively.

The enhancing effects of IBMX were the same as those observed with *B. superba*. IBMX, a non-specific phosphodiesterase inhibitor, relaxed the cavernous smooth muscle in a dose-dependent manner (10^{-8} - 10^{-4} M) (Figure 3B). IBMX at the concentration of 10^{-4} M almost completely relaxed the cavernous smooth muscle from normal control group. At the highest concentration studied (10^{-4} M) could induce only about 50% relaxation from diabetic control group. The EC_{50} were about 1.48 x 10^{-6}, 7.45 x 10^{-6}, 1.94 x 10^{-6}, 1.85 x 10^{-6} and 4.91 x 10^{-6} M from normal control, diabetic control, and treated group (with extract at the doses of 1, 10 and 100 mg/kg BW), respectively.

4 Discussion

Previous studies on erectile function in the STZ-induced diabetic rats have shown that this condition adversely affects the erectile capability, with a reduction in the ICP responses to nerve stimulation after only 2 - 3 months following the STZ treatment [18]. Erectile dysfunction is known to be a major health problem and can cause considerable distress, unhappiness and relationship problems. The treatment of ED, however, can be many faceted and may involve more than one regimen. Recently, several studies interested to treat ED with medicinal plants, which are being shown to be both effective and safe [9]. In this study, we interest to use *B. superba* that is promising "future candidates" for the treatment of ED in diabetic rats. It has long been used for treat impotence in Thai male [10]. The mechanism of *B. superba* to induce penile erection is not clearly known. But our previous data demonstrated that it enhanced penile erection and also increase the activity of cGMP or IBMX for relaxing

the smooth muscle [7]. This indicates that *B. superba* may act by inhibiting phosphodiesterase enzymes. When the phosphodiesterase was already inhibited, the relaxation observed were the results of the action of higher extracellular cGMP and of the unhydrolyzed endogenous cyclic nucleotides. This is correspondence with a previous study reporting that *B. superba* inhibits cAMP phosphodiesterase *in vitro [6]*. Although there is a strong relationship between relaxation of the corpus cavernosum smooth muscle and sexual function [11] , it is also well known that there are many other factors that contribute to the deterioration of sexual function [12].

The corpora cavernosa consists of the smooth muscle cells, loose areolar tissues, blood vessels, nerves and collagen fiber, which is essential for a penile erection and is implicated with ED. Smooth muscle cells play a critical role in corporal relaxation and the reduction can lead to an ED [13], [14]. From previous study, morphometric analysis of the sections showed a significant reduction in the density of cavernosal smooth muscle cells in diabetic animals [15]. The importance of the smooth muscle has been appreciated in both animal experiments and human clinical studies and alterations occurring in the structure of the different components in diabetes mellitus have been reported [16, 17]. Recently, it was reported that image analysis of the corpora cavernosa in the STZ rat model is a reliable and reproducible research tool for assessing the diabetes-related vascular damage [16].

Previous studies on erectile function in the STZ-induced diabetic rats have shown that this condition adversely affects the erectile capability, with a reduction in the ICP responses to nerve stimulation after only 2 - 3 months following the STZ treatment [18]. However, the evaluation of the effectiveness of *B. superba* in enhancing penile erection is still difficult. In this study, the result showed that a 4-week period of the STZ–induced diabetes caused a measurable and significant reduction in the ICP and

smooth muscle relaxation in corpora cavernosal with a loss of erectile function. In the rat model, ICP measurements have gained wide acceptance for evaluating erectile function because of the similarities between humans and rats with respect to erections [19].

The intracavernous pressure was monitored after the administration of *B. superba* extract. The ICP of the diabetic animals were significantly lower than in the control animals. However, the decrease in the ICP was significantly recovered by *B. superba* treatment. These results were comparable to the previous study, which demonstrates that diabetic rats consistently showed lower ICP in response to electrostimulation of cavernous nerve, physical manipulation and pharmacological stimulation [18]. In addition, the results show that the response followed the rule of dose-response relationship at the low extract concentrations (1-10 mg/kg BW) with the maximum effect at dose of 10 mg/kg BW. After then the increased ICP were reduced when the extract concentrations were increased (100 mg/kg BW). Regarding the effect of the extract, the result exhibited a bell shape response curve. This suggests that *B. superba* extracts may increase the ICP via unknown receptor-mediated mechanisms. Normally, a bell shape response curve can be observed in a receptor saturation phenomenon. The bell shaped form of the dose response curve is clearly not a consequence of the dependence of simple receptor binding by ligand. It may be that, at the equilibrium, the extracts may bind the receptors at multiple sites. Alcoholic extracts usually contain a number of compounds. This phenomenon could happen with the *B. superba* alcoholic extracts since at the higher doses the ICP enhancing activity of *B. superba* were decreased.

Penile erection is a result of an increase in ICP. Agents those are capable of increasing blood flow can also enhance the erection of the penis such as

nitrovasodilators (e.g. sodium nitroprusside) [20]. Vasodilators are usually used to rapidly decrease work load to the heart by decreasing blood pressure via dilating of the blood vessels. Therefore, it is possible that any ICP modulating agents are able to alter the MAP. Therefore, the effect of the *B. superba* extracts on the MAP in the rats was primarily investigates. The result shows that the MAP was not affected by the exposure to the extract. This indicates that the alcoholic extract of *B. superba* may no cause undesirable hypotensive effect.

This study further investigates for enhancing the relaxation of smooth muscle in corpora cavernosa of the alcoholic extract using an *in vitro* model. IBMX is a non-specific phosphodiesterase inhibitor and commonly used to increase the cyclic nucleotides levels *in vitro*. IBMX or in turn cyclic nucleotides including the exogenous cGMP relax the smooth muscle via cAMP and cGMP dependent kinase [21]. The result shows that extract relaxed the smooth muscle. Exogenous IBMX also relaxed the smooth muscle. The relaxations of the diabetic control were significantly lower than in the normal control. However, the decrease in the relaxation was significantly recovered by *B. superba* treatment. Although using ICP and cavernosal smooth muscle tone to evaluate penile erection is rather effective, the present study was not executed on cavernosal tissue animals. Further studies are required about immunohistochemistry of cavernosal tissue, to investigate changes of smooth muscle, endothelial cells, and fibrous tissue in crus of the penis, for providing valuable information.

In conclusion, this study indicates that the alcoholic extracts of *B. superba* can increase intracavernous pressure with the most effective dose of 10 mg/kg BW. It is also suggest that *B. superba* may act by enhancing relaxation of smooth muscle in the corpora cavernosa. These results were comparable to the

previous study, *B. superba* enhanced penile erection [7]. Moreover, acute toxicity study (5,000 mg/kg BW) showed that there was no mortality and *B. superba* at 250 mg/kg BW which was 100 times more than the Thai FDA recommended dose for humans appeared to be safe in rats [22, 23]. These results suggest that the treatment of alcoholic extracts of *B. superba* can partially prevent the development of ED experimentally induced by STZ administration, providing a rationale for the potential use of *B. superba* extracts for treating the ED secondary to diabetes mellitus.

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