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**Short Communication**

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# The Tonic Effect of the Extract from Male Silkworm (*Bombyx mori* L.) Pupae on Rats

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**More than three hundred million men in the world are suffering from erectile dysfunction. Korean ancient medical records say that the unmated male silkworm moth is effective in strengthening men's vitality, but no scientific examination has proved its clinical efficacy. Currently, silkworm moths are not allowed for foodstuff. Thus, we selected silkworm of 14-days-after-metamorphosis, at which external morphology possesses that of pupae, but internal component is that of moths. To obtain the reliable source of unmated male silkworm moth, we used the sex-limited silkworm breed with larval marking. The body marker allows casual separation of sex during larval period, preventing mating right after emergence from cocoon. Using the extract prepared with the pupae of 14-days-after-metamorphosis, we investigated the tonic effect of the extract on the rat fed the extract for three weeks. The results showed that the testosterone levels in serum increased maximum by 19%, that of testicle increased maximum by 200%, and athletic endurance of the rats rose by 6%, suggesting positive tonic effect of the pupae prepared at the stage of 14 days after metamorphosis.**

**Key words**: Silkworm, Silkworm pupae, Tonic effect, Testosterone, Erectile dysfunction

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## Introduction

Erectile dysfunction (ED), which affects millions of men is defined as the inability of a man to achieve or sustain an erection sufficient for his sexual needs or the needs of his partner. Most men experience this inability at some point in their lives, usually by age 40. Some men experience chronic, complete erectile dysfunction (impotence), and others achieve partial or brief erections.

Causes of erectile dysfunction are physiological and psychological. Reduced blood flow to the penis and nerve damage are the most common causes, although underlying causes are diversified (DasGupta and Fowler, 2002).

There are several ways to treat erectile dysfunction. These include oral medications, vacuum erection devices, injections, intra-urethral pellets and surgeries, depending on its symptom. Among these, oral medication includes Viagra (Morales *et al.*, 1998; Dinesha *et al.*, 2002) and Yohimbine (Tam *et al.*, 2001). In March 1998, the Food and Drug Administration of USA approved sildenafil citrate (marketed as Viagra), the first oral pill to treat impotence. Viagra has truly revolutionized the treatment of erectile dysfunction. It is relatively effective and safe in approximately 60% of men. Taken one hour before sexual activity, sildenafil works by enhancing the effects of nitric oxide, a chemical that relaxes smooth muscles in the penis during sexual stimulation, allowing increased blood flow. Yohimbine is another oral medication that predated Viagra. Its efficacy is controversial, although it is generally believed to produce modest improvement in erectile dysfunction. On the other hand, side effect of Viagra also has been reported substantially (Egan *et al.*, 2002; Tzathas *et al.*, 2002; Wysowski *et al.*, 2002).

In Korea, several Chinese medications have traditionally been utilized for the treatment of erectile dysfunction, but their effect has never been scientifically proved until recently. Korean ancient medical records, including Don-

geuibogam, the bible of Oriental medicine, say that the unmated male silkworm moth is effective in strengthening men's vitality, although detailed mechanism of the efficacy and methods for oral use are not available.

In this study, we fed rats the extraction of male silkworm pupae to test the tonic efficacy of the extract on the rats.

## Materials and Methods

### Preparation of unmated male silkworm moth and discrimination of sex

In the Korean ancient medical records, the erectile dysfunction of silkworm is mainly ascribed to the unmated male silkworm moths. However, only silkworm larvae and pupae are currently registered as food sources. Thus, the conflict should be overcome to commercialize when silkworm product shows the positive effect against erectile dysfunction. The difficulty was overcome by selecting the pupae at a stage when external morphology possesses that of pupae, but internal component in reality is that of moths. In a serial experiment to pick out such stage, it was found that the pupae of 14-days-after-metamorphosis were satisfactory for the criterion (data not shown). Fig. 1 shows the morphological changes during pupae period without external conversion into moth until 14-days-after-metamorphosis. The pupae of 14-days-after-metamorphosis were utilized as pupae sample to prepare pupae extract.

Separation of male and female pupae was performed by expertise on the basis of shape at the tail part of larvae (Fig. 2). According to the Korean ancient medical records, only unmated male moths are effective against erectile dysfunction. Although, technically possible, it is very labor-intensive, time-consuming, and even difficult to separate male and female moths right after emergence. Moreover, male moths show a vigorous sexual appetite, looking for female moths to mate immediately after they thrust out of cocoon and mating continues until they are exhausted. Fig. 2 shows the scene of mating. To overcome such problems as separation of sexes and prevention of mating, we used the sex-limited silkworm breed with larval marker. The breed shows body marker on the back of only female larvae (Fig. 4) and the marker is obvious enough for the beginners to separate female and male casually. Thus, we prepared silkworm larvae extract by using the breed.

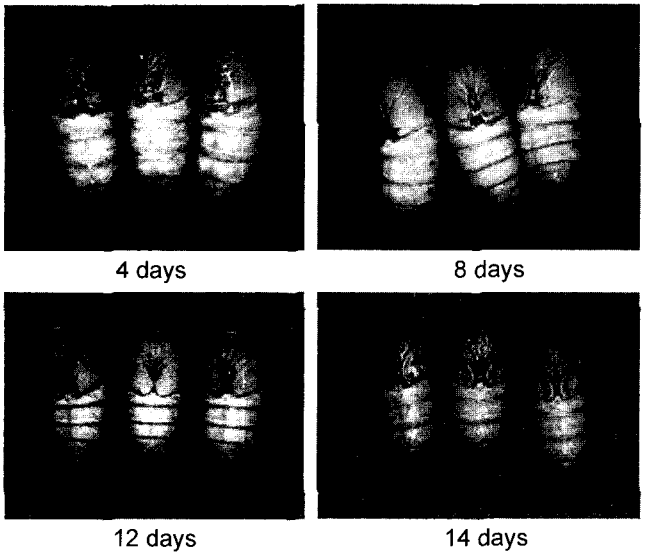


**Fig. 1.** Morphological change of silkworm pupae. Note that the external morphology of the pupae are remained as pupae until 14-days-after-metamorphosis.

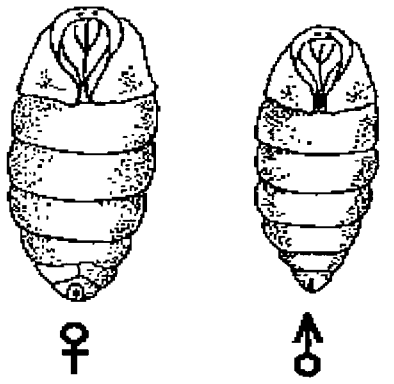

**Fig. 2.** Male and female silkworm pupae.

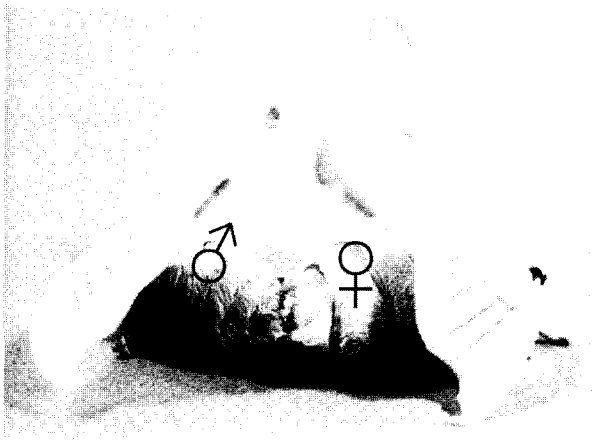

**Fig. 3.** A scene of mating.

### Preparation of silkworm extracts

Silkworms utilized for the extraction were reared in the Department of Sericulture and Entomology (NIAST) in June 2002. Properly separated unmated silkworm pupae

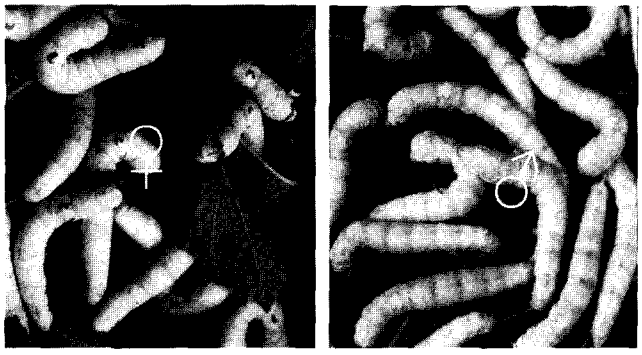

**Fig. 4.** The silkworm breed of sex-limited silkworm with larval marker on the the back of female silkworm larvae.

and moths were, respectively, frozen in the liquid nitrogen, freezing-dried, and powdered. Lyophilized powders were subjected to extraction with fifty-fold of ethanol at 60 for 1 h, followed by filteration with filter paper, and these steps were repeated for five times. The supernatants were pooled and freezing-dried to prepared rat diet.

### Animals

Four-week-old male mice of SD-strain were purchased from SLC Ltd. (Shizuoka, Japan) and were grown for additional 3–4 weeks. Mice were housed 5–6 animals to a cage and maintained at 23°C and 65% of room temperature and humidity, respectively (12 hrs of light-dark cycle) with free access to rodent diet (Sam Yuk Lab. Animal. Co. Ltd., Osan, South Korea) and tap water. When mice grew to weight of 250 ± 10 g, they were divided into 7 groups (n = 5 per group) and fed on diet of various additives for 3 weeks. The additive for each group is saline as a negative control. The blood serum and testes tissue were taken at fourth week.

### *Quantification of testosterone*

The level of testosterone was determined by o-phthaldehyde method. 0.1 mL of sample was added by 0.3 mL of KOH solution (33%, v/v) and 3.0 mL of ethanol (95%, v/v) and vortexed. Serum and testis samples were maintained at 60°C for 15 min and 60 min, respectively, and were cooled down in ice bath. Five milliliter of n-hexane and 3 mL of water were added and vortexed for 10 min. One milliliter of *n*-hexane (upper layer) was taken and concentrated by $N_2$ gas purge. Samples were added by 2 mL of *o*-phthaldehyde and 1 mL of concentrated $H_2SO_4$ and mixed well. Between 10 and 90 min after addition of $H_2SO_4$, absorbance at 550 nm was measured.

### Measurement of athletic endurance by forced swimming

Stamina was determined by the method described in Matsumoto *et al.* (1996). Male mice of ICR-strain were fed on various diet described above for 4 days, withdrawn for 24 hr, and forced to swim in the 100×50×50 cm of water bath. The time of submerging of mouse head beneath the water was recorded. Water was maintained at 14–16°C.

## Results and Discussion

### Comparison of testosterone content between the extracts from male moths and male pupae

Testosterone is the most important representative of the male sex hormones collectively called androgens. We compared the effect of the extracts from unmated male moths and male pupae. As explained above, we used pupae sample from the pupae of 14-days-after-metamorphosis. Thus, the pupae utilized in this study in fact are internally moths, with the appearance of pupae. On the other hand, we used moth extracts, by immediately collecting male moths, emerging from cocoons. In the rats fed 1% of silkworm moth extract, the testosterone level increased 15%, but the rat group fed 1% of silkworm pupae extract increased somewhat largely (19%) compared to the normal group, which fed saline only as an additive in the diet (Table 1).

**Table 1.** Comparison of testosterone contents in serum between the extracts from moths and pupae

| Treatments | | Testosterone content (mg/dl serum) | Percent increase (%) |
|---|---|---|---|
| Normal | | 26.78 | 100.0 |
| Unmated silkworm moth extract | 1.00% | 30.94 | 115.5 |
| | 0.10% | 30.40 | 113.5 |
| | 0.01% | 28.59 | 106.8 |
| Unmated silkworm pupae extract | 1.00% | 31.94 | 119.3 |
| | 0.10% | 30.58 | 114.2 |
| | 0.01% | 28.86 | 107.8 |

**Table 2.** Comparison of testosterone contents in testicles between the extracts from moths and pupae

| Treatments | | Testosterone content | Percent increase (%) |
|---|---|---|---|
| Normal | | 7.24 | 100.0 |
| Unmated silkworm moth extract | 1.00% | 13.57 | 187.4 |
| | 0.10% | 9.05 | 125.0 |
| | 0.01% | 4.52 | 62.4 |
| Unmated silkworm pupae extract | 1.00% | 21.72 | 300.0 |
| | 0.10% | 10.86 | 150.0 |
| | 0.01% | 9.05 | 125.0 |

**Table 3.** Test of stamina by forced swimming

| | Normal | Unmated silkworm moth extract | Unmated silkworm pupae extract | Powdery silkworm | Viagra | Tocopherol |
|---|---|---|---|---|---|---|
| Swimming time (min) | 14.42 ± 3.71 | 23.10 ± 8.71 | 15.33 ± 8.71 | 20.37 ± 8.71 | 12.16 ± 8.71 | 17.21 ± 8.71 |
| Percent increase (%) | 100 | 160 | 106 | 141 | 84 | 119 |

In the experiment to test the testicle testosterone content, the rat group fed 1% of silkworm pupae extract also showed larger increase (200%) than the group fed 1% of silkworm moth extract compared to the normal group (Table 2). Therefore, these results suggest that the unmated silkworm pupae of 14-days-after-metamorphosis can casually replace for the silkworm moths, which is currently not allowed for foodstuff.

**Test of athletic endurance by forced swimming**

Although the ability of forced swimming is not directly relevant to the erectile dysfunction, it has something to do with masculinity. Thus, we compared the ability of forced swimming of the rats fed several sources of silkworm products and Viagra and Tocopherol (Table 3). The most obvious increase was observed in the rat group fed moth extract (60%) and powdery silkworm (41%) next. All groups showed increased duration of forced swimming ability compared with the normal group.

In summary, we tested the efficacy of the pupae of 14-days-after-metamorphosis to test if the extract exerts tonic effect. The results showed obviously positive efficacy compared with control, although further systematic experiment is required for commercialization of the products containing the pupae of 14-days-after-metamorphosis as a major ingredient. The result of this experiment has been submitted for Korean patent.

## References

DasGupta, R. and C. J. Fowler (2002). Sexual and urological dysfunction in multiple sclerosis: better understanding and improved therapies. *Curr. Opin. Neurol.* **15**, 271-278.

Dinesha, N. D., B. K. Vishukumara, P. Nagarajaa, N. M. Made Gowdab and K. S. Rangappa (2002) Stability indicating RP-LC determination of sildenafil citrate (Viagra) in pure form and in pharmaceutical samples. *J. Pharm. Biomed. Anal.* **29**, 743-748.

Egan, R. A. H. Pomeranz, J. C. Morgan and K. C. Johnston (2002) Transient ischemic attack an stroke associated with sildenafil (viagra) use [2] (multiple letters). *Neurology* **59**, 293-294.

Matsumoto, K., K. Ishihara, K. Tanaka, K. Inoue and T. Fushiki. (1996) An adjustable current swimming pool for the evaluation of endurance capacity of Mice. *J. Appl. Physiol.* **81**, 1843-1849.

Morales, A., C. Gingell, M. Collins, P. A. Wicker and I. H. Osterloh (1998) Clinical safety of oral sildenafil citrate (VIAGRA) in the treatment of erectile dysfunction. *Int. J. Impot. Res.* **10**, 69-73.

Morrissey, R. E., B. A. Schwetz, J. C. Lamb, M. D. Ross, J. L. Teague, R. W. Morris (1988) Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies. *Fundam. Appl. Toxicol.* **11**, 343-358.

Tam, S. W., M. Worcel and M. Wyllie (2001) Yohimbine: a clinical review. *Pharmacol. Ther.* **91**, 215-243.

Tzathas, C., A. Christidou and S. D. Ladas (2002) Sildenafil (viagra) is a risk factor for acute variceal bleeding. *Am. J. Gastroenterol.* **97**, 1856.

Wysowski, D. K., E. Farinas and L. Swartz (2002) Comparison of reported and expected deaths in sildenafil (Viagra) users. *Am. J. Cardiol.* **89**, 1331-1334.