

## Testosterone Secretion Effect according to the Growth Stage of Silkworm (*Bombyx mori* L.)

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(Received 10 May 2010; Accepted 11 June 2010)

Until now, the main treatment for impotence has been the vasodilator injection, penile implants and etc. Among many methods, most effective way is known to be the medical therapy. The oral drug for an impotence remedy has to be above all, effective to the erection, and second, safety when taking in long-term. However, so far the drug in such condition and sufficiency has not been discovered. Consequently, it is crucial to develop the new medicine, made of natural materials only, without any uncertainty of side effect. The silkworm is very difference in physiological chemical change according to growth stage. Therefore the functional effect shows a very big difference according to preparation method with a growth stage. This research carry out the experiment with the pupa powder immediately before the eclosion, eclosion adult, the pupa extract immediately before the eclosion, and the silkworm powder of 5 instar 3rd day. The result showed not a big chance in weight, feed and water intake quantity. But testosterone hormone secretion effects of the freeze drying powder of the pupa immediately before the eclosion was very high 7.31 ng/ml, compared to 2.37 ng/ml of non treatment and 2.67 ng/ml of alcohol extraction method. As this result, the freeze drying pupa powder of the pupa immediately before the eclosion was confirmed to promote the secretion of the testosterone hormone more than alcohol extraction method of existing.

**Key words:** Silkworm, Silkworm pupae, Testosterone Hormone, Erection

### Introduction

Erection dysfunction, 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. Causes of erectile dysfunction are physiological and psychological. Reduced blood flows to the penis and nerve damage are the most common causes, although underlying causes are diversified (Das Gupta 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. 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 erection dysfunction, but their effect has never been scientifically proved until recently. Korean ancient medical records, including Donggeuibogam, 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. The alcohol extract of male pupae 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. (Ryu *et al.*, 2002)

In this study, we fed SD rats the silkworm powder of 5 instar 3rd day, the silkworm powder of spinning start, the

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pupae powder immediately before the eclosion, eclosion adult powder, the pupa alcohol extract immediately before the eclosion to test the tonic efficacy on the rats.

## Materials and Methods

### Preparation of silkworm according to the growth stage

The silkworms used for the experiment were Yang-wonjam that are successively preserved as the silkworm leading variety in Korea. This variety is sex-limited silkworm breed with larval marker. This variety shows body marker on the back of only female larvae and the marker are obvious enough for the beginners to separate female and male casually. This silkworm was reared in spring season at 2009 in National Academy Agricultural Science. The 5th instar 3rd day larvae and spinning start larvae were quickly frozen with the liquid nitrogen and the pupae immediately before the eclosion, eclosion adult were frozen in deep freezer. These samples make fine powder with grinder mixer machine.

### Preparation of silkworm extracts

Silkworms utilized for the extraction were reared at 2009 in National Academy Agricultural Science. Properly separated unmated silkworm pupae were frozen in the deep freezer and ground into powdered. Lyophilized powders were subjected to extraction with fifty-fold of ethanol at 60°C for 1 h, followed by filtration with filter paper, and these steps were repeated for five times. The supernatants were pooled and freezing-dried to prepared rat diet.

### Animals

Six-week-old male mice of SD-strain were purchased from SAMTAKO Bio Korea and were grown. Rats reared 5 animals to a cage and maintained at 22°C and 50% of room temperature and humidity, respectively (12 hrs of light and dark cycle) with free access to rodent diet (SAMTAKO Bio Korea) and sterilized water.

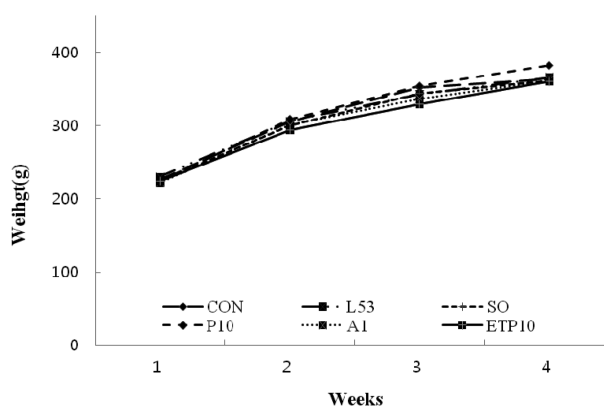
When rats grew to weight of 210~240 g, they were divided into 6 groups (n=5 per group) and fed on diet of various additives for 4 weeks as Table 1. The blood serum was taken after fasting for 16 hours before the end of experiment.

### Quantification of testosterone

The rats were fasted for 18 hours before autopsy. The blood serum was taken at abdominal vein after incised the abdomen with ether treatment. This serum was separated by centrifuge at 2500 rpm, 15 min. The level of testosterone was analyzed at UB Medical Institute.

**Table 1.** The diet of various sample additives

Treatment	Rats	Diet
Control(Con)	5	Normal diet
5instar 3 <sup>rd</sup> days larvae(L53)	5	Mixed 0.7%
Spinning start(SO)	5	Mixed 0.7%
pupae before the eclosion (P10)	5	Mixed 0.7%
eclosion adult (A1)	5	Mixed 0.7%
alcohol extract of pupae(ETP10)	5	Mixed 0.2%
Total 6 groups	30 animals	



**Fig. 1.** Body weight changes in rats fed diets of the silkworm for 4 weeks.

## Results and Discussions

### Change in body weight

Our analysis focused on the comparison of change in body weights between the treatment group and the control group. The result didn't show much difference between two groups regardless of time changes (Fig. 1). There is not apparent weight loss of the control group which proves that the nutrition was not a relative matter.

The silkworm sample according to the growth stage is very safe in animal test.

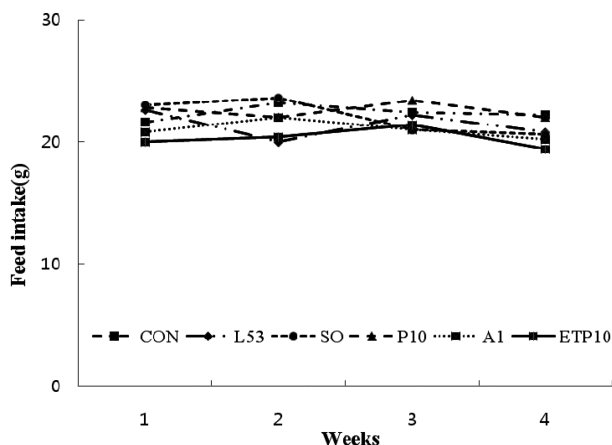
### Change of feed intake

The daily diet for the experiments was 200 g per cage. After 24 hours of food supply we subtract the remainder from 200 g per cage. Then, we divided the amount by the number of animals in the cage to obtain the quantity of feed intake. The result shows no significant difference in feed intake between the groups (Fig. 2).

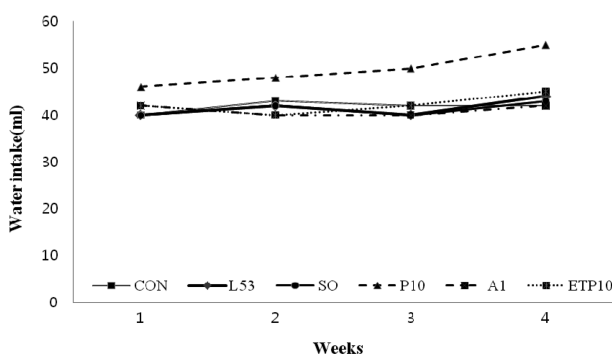
This is considered with the fact that does not have a big problem to feed which adds the silkworm samples and also the material, which the animals don't dislike the thing.

### Change of water intake in rats

We prepared 500 ml water the day before water intake for



**Fig. 2.** Change in feed intake in rats fed diets of the silkworm for 4 weeks.

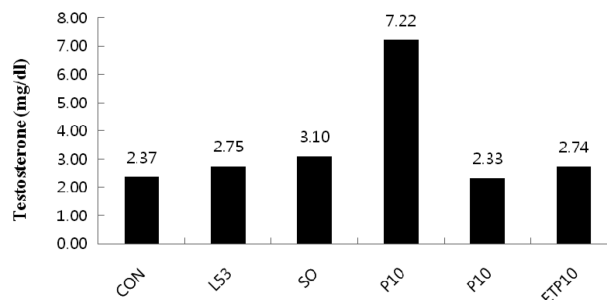


**Fig. 3.** Change in water intake in rats fed diets of the silkworm for 4 weeks.

water intake quantity. The remainder of water after 24 hours from given water 500 ml leaves the water intake quantity. The water intake quantity of each rat is value which divided the total water intake quantity per animals of cage. Among the treatments, the pupae powder immediately before the eclosion in took the much water (Fig. 3). But the difference is non-significant.

### Serum testosterone level

We compared the testosterone secretion effect of the lyophilized powders and extracts from silkworm larvae, male pupae and unmated male moths. As explained above, we used pupae sample from pupae immediately before the eclosion. Thus, the pupae utilized in this study in fact are internally moths, with the appearance of pupae. In the rats fed 0.2% of pupae extract, but the other sample fed 0.7% with powder. Among these samples, the powder of from pupae immediately before the eclosion showed larger



**Fig. 4.** Serum testosterone level of rats fed sample diets for 4 weeks.

increase (308%) than the normal group, which fed the normal diet without silkworm powder (Fig. 4). As this result, the freeze drying pupa powder was confirmed to promote the secretion of the testosterone hormone more than alcohol extraction method of existing. The result of this experiment has been submitted for Korean patent.

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