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Effects of Black Pepper (*Piper nigrum* Linn.) Extract on Sexual Drive in Male Mice

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ABSTRACT

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Black pepper (*Piper nigrum* L.), known as the king of spices, proven to contain various types of active substances that are allegedly beneficial to the human body functions including sexual function. This study was conducted to determine whether black pepper extract can be used to improve sexual function in male subjects. Thirty six healthy male mice, four months old, weighing between 25-30 g, were used as experimental animals and grouped into four. The first one mice was given pellets that do not contain black pepper extract as a control. The second and third groups, respectively were treated with pellets containing aqueous and ethanol extracts. The last group was given pellets containing aqueous extract and ethanolic extracts with a ratio of 1:1. Pellets were given once every day for 90 days. Mating behavior test was conducted in an open round plastic tray with a diameter of 40 cm and height 25 cm. Throughout the test, video taping was performed for the following parameters: courtship latency and mounting frequency. The results revealed that compared with control group, male mice fed on aqueous as well as ethanol extract of black pepper significantly showed a shorter courtship latency ($p < 0.05$). On the other hand, there was no difference in the mounting frequency between treated groups. Interestingly, the mounting frequency showed a strong negative correlation with the courtship latency ($r = -0.968$). In conclusion, the fruit extract of black pepper potentially affect sexual drive in male mice.

Key words: *Piper nigrum*, black pepper, sexual drive, mating behavior

INTRODUCTION

Nowadays, it can be easily found, advertisements on the internet offering supplement containing pepper extract with various labels such as: Piperine, BioPerine, Xbrain, Vitacost, Naturalin etc. Some advertisements claim that the concentrated extract of black pepper can be used to improve sexual function of men. To convince that the efficacies of the products have been scientifically proven, most of the ads are equipped with scientific citation, including Indian ancient texts such as Ayurveda (Mishra, 2012).

The biological role of piperine which is commonly known is antioxidant, anticancer, antipyretic, anti-inflammatory, anti-microbial agent and many more (Ahmad *et al.*, 2012). Another role of piperine which most widely associated with health improvement is its function as bioenhancer. As bioenhancer, piperine can enhance the bioavailability of companion drugs either by inhibiting the drug metabolizing enzyme or by enhancing the permeability of intestinal mucosa and hence results in higher plasma levels of drugs (Acharya *et al.*, 2012).

But on the other hand, many studies even show conflicting results. Based on the study about yaji (a typical herbal of Africa containing pepper), Nwaopara *et al.* (2009) found that high doses of these ingredients can induce brain tissue damage and tumor formation in rats. Next, Khani *et al.* (2013) reported that petroleum ether extract of *Piper nigrum* and *Jatropha curcas* at lowest concentration showed strong inhibition on egg hatchabilities and adult emergence of rice moth (*Corcyra cephalonica*). In addition, Bassey *et al.* (2011) reported that consumption of crushed seed of black pepper continuously for seven days even when used with minimum doses may be detrimental to health.

In connection with reproductive function, black pepper extract effects on human and animal seem to contradict each other as well. Kpomah *et al.* (2012), for instance, found albino male rats (*Rattus norvegicus*) treated with diherbal mixture of *Zanthoxylum leprieurii* and *Piper guineense* showed significant increase in libido parameters. From Indonesia, Rahmawati and Bachri (2012) also reported that male rats treated with a combined extracts of *Piper retrofractum*, *Centella asiatica* and *Curcuma domestica* showed a significant increase in sexual drive. However, there is concerned that piperine may have a negative impact on fertility. As found by Mishra and Singh (2009), male mice treated orally with black pepper powder significantly shows antispermatogenic and antifertility effects. Furthermore, piperine also could allegedly damage the epididymal environment and sperm function due to its ability to increase the reactive oxygen species levels by lowering the activity of antioxidant enzymes and sialic acid levels in the epididymis (D'Cruz and Mathur, 2005).

However, given the source of piperine used and discussed above not solely derived from *Piper nigrum*, it is necessary to investigate the effects of piperine of *Piper nigrum* only and directly. Supposing piperine from *Piper guineense* (Kpomah *et al.*, 2012) or *Piper retrofractum* (Rahmawati and Bachri, 2012) is the key factor responsible for sexual arousal in rats, the same active substance from *Piper nigrum* (black pepper) can also reveals similar phenomenon in mice.

MATERIALS AND METHODS

Extraction of the spices: In this study, two types of black pepper extract, ethanolic and aqueous, were prepared. All extraction steps initiated with grinding the black pepper fruits into powder form. Ethanolic extract was made by soaking the black pepper powder in 95% ethanol in room temperature. The supernatant collected every 24 h for three days and evaporated under low pressure until the brownish-viscous extract formed (Ismail *et al.*, 2012). To make aqueous extract, the black pepper powder was added to the boiling distilled water for 15 min and after filtering the solvent evaporated until the extract became a pasta form (Naseri and Yahyavi, 2007).

The pasta of the extract then mixed completely with the pellets formulated specifically for mice that had been re-grinded. The amount of black pepper extract added to the pellet was based on the tolerated dose limit of Trikatu, a generic Ayurvedic medicine prepared by mixing fruit powder of black pepper (*Piper nigrum* Linn.), fruits of long pepper (*Piper longum* Linn.) and rhizomes of ginger (*Zingiber officinalis* Rosc.) in the ratio of 1:1:1 which is 5 mg/kg body weight (Chanda *et al.*, 2009).

Given Trikatu consist of three species, two peppers and one ginger, so, that the tolerated limit of piperine dose should be 3.33 mg. Referring to the safe dose mentioned above, a single dose of piperine for mice with an average weight of 30 g supposedly was 0.1 mg/mouse.

Assuming each mouse needs food as much as 10% of its body weight, per day, mice with an average weight of 30 g would require food as much as 3 g. In order to make the food always

available (*ad libitum*), the amount of pellets prepared for each mouse was rounded up to 4 g daily. To meet the daily dosage, hence to the every 1 kg of pellet was added extracts of black pepper of 25 mg.

Next, the mixture was re-molded and re-dried to become a renewed pellet. The renewed pellet, then, served for treating the experimental mice.

Experimental animals and treatments: Male Swiss albino mice aged of 4 months and weight of 25-30 g from Lampung Veterinary Center, Indonesia, used. Thirty six male mice divided into 4 groups consisted of 9 mice each. The first group was given the pellet that did not contain extracts of black pepper as the control. The second and third groups were consecutively treated with pellet containing aqueous and ethanolic extract. While the last one is mice which fed with pellets containing a mixture of aqueous and ethanolic extracts of black pepper with a ratio of 1:1. The combined extracts were made by adding aqueous and ethanolic extracts of 12.5 mg each into 1 kg of pellets. Each mouse was caged individually and received 10 g of pellets containing extract and water *ad libitum* daily for 90 days.

Mating behavior test was carried out in an open round plastic tray with a diameter of 40 cm and height 25 cm. To begin the test, a treated male was allowed to adapt the tray environment for at least 5 min before an estrous female introduced. Starting from an estrous female put into the tray mating activities of the mice then observed for 30 min.

Throughout the test, videotaping was performed for the following parameters: Courtship latency and mounting frequency. Courtship Latency (CL) was the period of time since female mice introduced into the tray until the male showed a courtship action for the first time. While Mounting Frequency (MF) was the total attempt made by male to ride on the females back during 30 min observation (Clancy *et al.*, 1984).

Statistical analysis: The data, presented as Mean±SEM, was analyzed using one-way analysis of variance (ANOVA). Where a significant difference was detected by ANOVA, the treated groups were compared with the control one using Tukey HSD test. In order to check if the data of Courtship Latency (CL) correlated to the data of Mount Frequency (MF), Vassarstats for linear correlation analysis was applied.

RESULTS

The effects of powder extract of black pepper on the mating behavior of male mice presented in Table 1. The one way ANOVA of the data on Table 1 revealed that there was a significant difference between groups ($p = 0.0205$). When compared to the control group using Tukey HSD test, the male mice that treated with aqueous as well as ethanolic extract of black pepper

Table 1: Effects of black pepper fruits extract on male mice

Variables	Control	Aqueous extract	Ethanolic extract	Combined extract	ANOVA	
					F	p
CL (second)	43.77±5.41	27.77±2.66*	28.22±3.68*	31.22±3.20	3.75	0.020
MF	1.11±0.38	3.78±1.07	3.22±0.89	2.44±0.83	1.90	0.154

*Significantly different ($p < 0.05$) compared to control group using Tukey HSD Test, CL: Courtship latency and MF: Mount frequency

2 significantly showed a decrease in courtship latency ($p < 0.05$). However, the results of one way ANOVA of mounting frequency showed no significant difference among treatment groups.

DISCUSSION

As shown in Table 1, male mice fed with black pepper extracts (the aqueous, the ethanolic and the 1:1 combination of aqueous and ethanolic) showed no significant difference in the parameters of mounting frequency compared to that of controls. However, when the data of mounting frequency and courtship latency tested using Vassarstats for linear correlation analysis, the results as shown in Table 2 are obtained. Interestingly, there was a strong negative correlation ($r = -0.968$, $p < 0.05$) between the data of mounting frequency and that of courtship latency.

Given a higher sexual drive usually and should be marked by a shorter courtship latency and a higher mounting frequency, hence the strong-negative value of r (-0.968) between two variables of sexual drive in question supposing that the extract of black pepper has potential effects on the sexual arousal in male mice. There are several possibilities that could explain the effects of piperine on sexual drive in animals.

The first possibility is, as suggested by Vijayakumar and Nalini (2006), that piperine supplementation might increase the plasma testosterone level. Testosterone, as had been known, is a hormone that responsible for male sexual libido (Wallen, 2001). Piperine is one of the constituents of *Piper nigrum* which have testosterone 5 α -reductase inhibitory effects due to its capability to catalyze the conversion of testosterone (Hirata *et al.*, 2007). The inhibitory effect of this enzyme causes testosterone levels remain high and thus libido has remained high.

The second possibility that make high sexual drive in male mice which fed on the extract may be related to the high content of fatty acids in black pepper. As shown by Meghwal and Goswami (2012), black pepper fruit is rich in auric acid, myristic acid and palmitic acid. As summarized by Gromadzka-Ostrowska (2006) in a review article, fatty acids may significantly affect the secretion and metabolism of androgens.

The next possibility, as can be read in many other literatures, black pepper is known to contain a variety of nutrients, including zinc (Nkansah and Amoako, 2010; Hamza *et al.*, 2007). Zinc, as reported by some researchers, is responsible for numerous physiological processes in organisms. Kumar *et al.* (2013) wrote that zinc supplementation can improve the antioxidative status and hormone levels in goat. In male rats, zinc supplementation had proved to increase serum levels of sex hormones including testosterone (Egwurugwu *et al.*, 2013).

In men, zinc was thought to contribute to fertility through its effects on semen parameters including sperm count (Ali *et al.*, 2007). Deficiency of this mineral can lead to reduce sexual drive in men, so that Sawidis *et al.* (2010) suggested that zinc is a key role in male sexual function.

Table 2: Results of correlation analysis between courtship latency and mounting frequency of experimental mice

Variables	Values
r	-0.968
r ²	0.937
df	2
t	-5.454
p-value	
One tail	0.016
Two tail	0.032

Beside the high content of zinc (Zn), black pepper also contains magnesium (Mg) in a significantly high concentration (Bouba *et al.*, 2012). It was found that Zn-Mg formulation significantly increase free testosterone levels in strength-trained, competitive athletes (Brilla and Conte, 2000).

CONCLUSION

Based on the data presented and discussed above it can be concluded that the fruit extract of black pepper potentially affect sexual drive in male mice.

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