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The Effect of Black Pepper Fruits (*Piper nigrum* L.) on the Increase of Erection

[1st] Nandia Septiyorini
North Purwokerto Health Center II

[2nd] Setyo Purwono
Department of Pharmacology, Faculty of Medicine Public Head and Nursing, Gajah Mada University

[3rd] Dicky Moch Rizal
Department of Physiology Faculty of Medicine Public Head and Nursing Gajah Mada University drdickyandrologi@ugm.ac.id

***Abstract*--Erectile dysfunction is the one of male sexual problems. Testosterone stimulate erection process physiologically. The study showed that black pepper extract increased sexual performance and testosterone level in animal research. The purpose of this study was to know the erotogenic effect, testosterone serum and concentration of brain androgen receptor of ethanolic extract of black pepper fruits. 25 adult male Wistar rats were divided into 5 groups: Na-CMC orally, TU/testosterone undecanoate (Andriol® Testocaps™) 4 mg/kg of body weight (BW) orally, the three groups were given of ethanolic extract of P. nigrum 3.33 mg, 6.66 mg and 13.32 mg/kg BW respectively for 55 days. Penile rat erection were observed by Total Penile Reflex (TPR). The measurement of testosterone serum and brain androgen receptor concentration used was ELISA method.**

Erection capability in the TU, P. nigrum 3.33 mg, 6.66 mg and 13.32 mg groups were increased until the 5th week observation and decreased after that. Na-CMC group erection capability increased gradually from 1st week until 8th week observation. Testosterone level in the Na-CMC and P. nigrum 6.66 mg decrease continually in the 4th and 8th week measurement. In TU group, P. nigrum 3.33 mg and 13.32 mg groups of testosterone level were increased in the 4th week and decreased in the 8th week. Brain androgen receptor concentration of the TU, P. nigrum 3.33 mg, 6.66 mg and 13.32 mg groups were lower than Na-CMC group. Extract of black pepper fruit had erotogenic effect on the increase of testosterone level of male Wistar rats.

Keywords: black pepper, erection, testosterone, androgen receptor

I. INTRODUCTION

Erectile dysfunction and decreased libido are the common male sexual health problems. Erection process is a combination of vascular, psychological, neurological and hormonal factors [1]. Testosterone as the main hormone in man, plays an important role in every step of the male sexual response [2]. Black pepper is one of the plants used to treat health problems including reproductive health problems.

Preliminary study showed that black pepper extract positively affected the sexual drive and increased testosterone level in male mice [3-4].

Piperin, a major alkaloid component of black pepper, has been reported to have potency in protecting nitric oxide from reactive oxygen species and increasing endothelial nitric oxide activity that has an important role in the erection mechanism [5]. Flavonoid, the other component in black pepper was proved to increase StAR gene expression in Leydig male mice cells that involved in cholesterol transport as a source of synthesis of testosterone [6]. Most of the action of androgen occurs through intracellular mechanisms involving androgen receptors [7]. The highest concentrations of androgen receptors in brain tissue are in the medial preoptic area (MPOA) of the hypothalamus as the sexual behavior control center [8-9]. Based on the advantages, the study on the effect of extrat on erection capability, serum testosterone level and brain receptor androgen concentration was necessary to be conducted.

II. METHODS

A. Experimental Design

An experimental in vivo study with pre-posttest with control group design were applied in 25 adult male Wistar rats that were divided into 5 groups.

B. Plant Extract

Dried fruit of black pepper was obtained from farmer in Lampung, determined by Department of Pharmaceutical Biology, Faculty of Pharmacy, UGM to make sure its species. Extraction was conducted at research laboratory, Department of Pharmaceutical Biology, Faculty of Pharmacy, UGM by maceration technique using 96% ethanol solvent [3].

C. Experimental animal

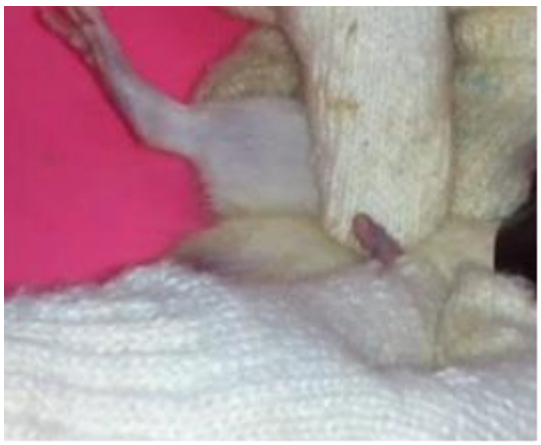
Experimental animals used in this study were 25 Adult male rats (*Rattus novergicus*) Wistar strain. Maintenance of experimental animals was carried out at the Pharmacology and Toxicology Laboratory, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, UGM, which was previously adapted in a physiological cage for 7 days. Rats were kept in a room temperature of 20-26^{o}C with a humidity of 40-70% and a dark-light cycle for 12 hours each. In 1 cage there were 34 mice. The food was given in the form of BR2 (*pur* as chicken feed) and given drink of distilled water ad libitum.

Adult male Wistar rats were divided into 5 groups; Na-CMC group were orally administered Na-CMC solution, TU group were given Testosterone Undecanoate (Andriol® Testocaps™) 4 mg/kg body weight (BW), *P. nigrum* 3.33 mg, *P. nigrum* 6.66 mg and *P. nigrum* 13.32 mg group were orally administered extract at the dose 3.33 mg/kg body weight, 6.66 mg/kg body weight and 13.32 mg/kg body weight respectively for 55 days. This study had been approved by ethic commission of LPPT-UGM with the certificate number 00050/04/LPPT/IV/2017.

D. Erection Capability Observation

The rats were mantained in supine position for 15 minutes. Penile sheath was retracted to see the erection response of the penis. Total penile reflex was recorded based on the accumulation of erection, quick flip and long flip frequency.

Picture 1. Erection (Glans penis become red and bigger in the basal of it)



Picture 2. Quick flip (Dorsiflexion of penile body <90º)

Picture 3. Long flip (Dorsiflexion of penile body 90º)

E. Hormonal Assay

Blood collected from orbital sinus was allowed to clot for 2 hours at room temperature. Blood samples were centrifuged at 2000-3000 RPM for 20 minutes. Supernatant was collected and stored at -80 ºC for future examination using ELISA technique based on its manual procedure (E-EL-0072, elabscience Co., Wuhan, China).

F. Concentration Assay of Androgen Receptor

At the end of experiment, rats were anesthesized by ketamine (Ketamine Hameln, 50 mg/mL, Hameln Pharmaceuticals, Germany) injected at the dose of 80 mg/body weight. Rats were sacrificed using decapitation technique to collect the brain tissue. Brain tissues were rinsed to remove excess blood thoroughly and weighed before homogenization. Homogenization was done in PBS (pH 7.4) with a glass homogenizer on ice. Homogenized tissue then sonicated for future lysis membrane and centrifuged at 2000-3000 RPM for 20 minutes. Supernatant was collected and stored at -20 ºC for future ELISA (E0909Ra, bt-laboratory, Shanghai Korain Biotech. Co Ltd) examination.

G. Statistical Analysis

Results were described as mean±SD. The significance difference between groups in every observation and examination period was tested by Oneway ANOVA. The significance difference between each observation and examination period in every group, was tested by repeated ANOVA.

III. RESULTS

A. Effect of Ethanolic Extract of Black Pepper on Body Weight

Table 1 showed that weight gain percentage from 4th to 8th week in the *P. nigrum* 6.66 mg and *P. nigrum* 13.32 mg groups was significantly lower than Na-CMC, TU and *P. nigrum* 3.33 mg groups (p=0.000).

TABEL 1. EFFECT OF ETHANOLIC EXTRACT OF BLACK PEPPER FRUITS (*Piper nigrum* L.) ON BODY WEIGHT GAIN (n=25)

Groups	Body weight (mean (g) ± SD)				
	Initial body weight (g)	4th week body weight (g)	8th week body weight (g)	Body weight gain initial – 4th week (%)	Body weight gain 4th – 8th week (%)
Na-CMC	202,20 ± 21,89	257,40 ± 33,80	283,80 ± 19,42	28,84 ± 12,48	11,45 ± 13,35
TU	206,30 ± 22,8	246,10 ± 39,65	304,40 ± 39,89	32,23 ± 18,80	24,28 ± 6,87
P. nigrum 3.33 mg	225,40 ± 12,50	272,00 ± 24,64	298,40 ± 20,62	20,67 ± 8,59	10,78 ± 16,06
P. nigrum 6.66 mg	241,20 ± 21,55	290,20 ± 28,74	238,20 ± 22,27	20,34 ± 6,38	-16,84 ± 14,76*
P.nigrum 13.32 mg	209,20 ± 28,19	264,40 ± 22,83	233,40 ± 29,45	27,43 ± 12,87	-11,73 ± 8,48*

TU: Testosterone undecanoate 4 mg/kg body weight group; *: weight gain percentage at 4th week untuil 8th week in kelompok *P. nigrum* 6.66 mg and *P. nigrum* 13.32 mg groups were significantly different (p<0,05) than Na-CMC, TUand*P. nigrum* 3.33 mg groups analyzed by Oneway ANOVA.

B. *Effect of Ethanolic Extract of Black Pepper on Erection Capability*

Table 2 showed the frequency of TPR in every groups. Na-CMC group showed an increase in TPR frequency in every week of observation. TPR frequency in the TU, *P. nigrum* 3.33 mg, *P. nigrum* 6.66 mg and *P. nigrum* 13.32 mg groups increased from 1st week observation to 5th week observation and decreased in the next week observation.

TABLE 2. EFFECT OF ETHANOLIC EXTRACT OF BLACK PEPPER FRUITS (*Piper nigrum* L.) ON ADULT MALE WISTAR RAT SERECTION CAPABILITY BASED ON FREQUENCY OF TOTAL PENILE REFLEX (TPR) (n=25)

Week of observ ation	Groups (mean± SD)				
	Na-CMC	TU	P. nigrum 3.33 mg	P. nigrum 6.66 mg	P. nigrum 13.32 mg
1	11,00 ± 2,00[c]	16,40 ± 2,70	13,80 ± 1,48	14,20 ± 1,92	13,60 ± 4,56
2	12,00 ± 3,08	17,60 ± 1,81	15,20 ± 2,16	16,20 ± 2,94	15,20 ± 3,70
3	13,00 ± 3,46[a]	19,20 ± 2,16	17,20 ± 2,77	15,40 ± 2,19	16,00 ± 2,23
4	14,00 ± 2,34[b]	21,00 ± 2,91[d]	18,00 ± 3,53	18,00 ± 2,54	18,60 ± 2,30
5	15,80 ± 2,77[b]	22,20 ± 2,28	19,00 ± 3,16	19,80 ± 2,28[f]	19,60 ± 1,14[g]
6	16,40 ± 2,30	19,60 ± 2,70	18,20 ± 1,64	16,80 ± 1,30	15,60 ± 2,30
7	16,80 ± 2,04	18,20 ± 3,89	16,00 ± 5,56	14,40 ± 2,88	14,40 ± 2,30
8	17,20 ± 2,58	16,60 ± 3,13	14,20 ± 4,08 [e]	12,80 ± 1,64	12,60 ± 1,67

TU: Testosterone undecanoate 4 mg/kg body weight group; a: TPR frequency of Na-CMC group significantly difference (p<0,05) than TU and *P nigrum* 3.33 mg groups analyzed by One way ANOVA; b: TPR frequency of Na-CMC group significantly difference (p<0,05) thanTU, *P. nigrum* 3.33 mg, *P. nigrum* 6.66 mg, and *P. nigrum* 13.32 mg analyzed by One way ANOVA; c: TPR frequency of Na-CMC group in the 1st week significantly difference (p<0,05) than 5th, 6th, 7th and 8th week analyzed by repeated ANOVA; d: TPR frequency of TU group in the 4th week significantly difference (p<0,05) than 1st, 2nd, 3rd, 5th, 6th, 7th and 8th week analyzed by repeated ANOVA; e: TPR frequency of*P. nigrum* 3.33 mg group in the 8th week significantly difference (p<0,05) than 4th, 5th and 6th week analyzed by Friedman test; f: TPR frequency of*P. nigrum*6.66 mg in the 5th weeksignificantly difference (p<0,05) than 1st and 8th week analyzed by Friedman test; g: TPR frequency of *P. nigrum* 13.32 mg group in the 5th week weeksignificantly difference (p<0,05) than 3rd, 6th and 7th week analyzed by repeated ANOVA.

C. *Effect of Ethanolic Extract of Black Pepper on Serum Testosterone Level*

Table 3 showed the level of testosteron in every group at week 0, 4 and 8 examination. Testosterone levels in the Na-CMC group in 3 times measurement did not differ significantly (p = 0.626), but their levels decreased between measurements. Testosterone levels of TU group at week 0 examination were significantly lower than week 4 (p = 0.003) and significantly higher compared to week 8 (p = 0.001).

Testosterone level of TU group at week 4 was also significantly higher than week 8 (p=0.013). Testosterone level of P. nigrum 3.33 mg group was significantly lower in the week8 compared to week 0 (p=0.038) and week4 (p=0.035). Testosterone level of P. nigrum 6.66 mg group in the week 8 was significantly lower compared to week 0 (p=0.011) and week 4 (p=0.006) level. In the P. nigrum 13.32 mg group, testosterone level in the week 8 of examination was significantly lower compared to week 0 (p=0.031).

TABLE 3. EFFECT OF ETHANOLIC EXTRACT OF BLACK PEPPER FRUITS (*Piper nigrum* L.) ON TESTOSTERONE SERUM LEVEL (n=25)

Groups	Serum testosterone level (ng/mL) (mean ± SD)		
	Week 0	Week 4	Week 8
Na-CMC	8,22 ± 1,64	7,58 ± 0,94	6,85 ± 2,25
TU	8,22 ± 1,39[b]	11,96 ± 3,05[a,c]	3,91 ± 1,06
P. nigrum 3.33 mg	7,90 ± 3,60	9,78 ± 1,77	4,86 ± 2,10[d]
P. nigrum 6.66 mg	8,96± 2,00	8,59 ± 2,07	3,89 ± 0,85 [d]
P. nigrum 13.32 mg	7,91 ± 1,66	8,17 ± 2,88	4,44 ± 1,00 [e]

TU: Testosterone undecanoate 4 mg/kg body weight group; a: testosterone level in the TU group significantly different (p<0,05) than Na-CMC group, *P. nigrum* 6.66 mg and *P. nigrum* 13.32 mg in the week 4 analyzed by One way ANOVA; b: testosterone level in the TU group in the week 0 significantly different (p<0,05) than week 4 and 8 analyzed by *repeated* ANOVA; c: testosterone level in the TU group in the week 4 significantly different (p<0,05) than week 8 analyzed by *repeated* ANOVA; d: testosterone level in the *P. nigrum* 3.33 mg and *P. nigrum* 6.66 mg groups in the week 8 significantly different (p<0,05) than week 0 and week 4 analyzed by *repeated* ANOVA; e: testosterone level in the *P. Nigrum* 13.32 mg group in the week 8 significantly different (p<0,05) than week 0 analyzed by *repeated* ANOVA.

D. *Effect of Ethanolic Extract of Black Pepper on Brain Androgen Receptor Concentration*

The concentration of brain tissue androgen receptor can be seen in Table 4. From the statistical analysis, it was obtained p=0.126 so it can be concluded that there was no statistically significant differences among groups. However, from the average concentration in each group, the Na-CMC group had the highest concentration compared to the other groups.

TABLE 5. EFFECT OF ETHANOLIC EXTRACT OF BLACK PEPPER FRUITS (*Piper nigrum* L.) ON BRAIN ANDROGEN RECEPTOR CONCENTRATION (ng/mL) (n=25)

Groups	Brain androgen receptor concentration (ng/mL) (mean ± SD)
Na-CMC	10,86 ± 1,62
TU	8,65 ± 2,34
P. nigrum 3.33 mg	8,72 ± 1,64
P. nigrum 6.66 mg	7,69 ± 2,37
P. nigrum 13.32 mg	8,12 ± 1,27

TU: Testosterone undecanoate 4 mg/kg body weight group; mean± SD analyzed by Oneway ANOVA

IV. DISCUSSION

Piperine significantly reduces serum triglyceride, total cholesterol, LDL and VLDL, increase serum HDL levels so it can be reduced the incidence of dyslipidemia. The results showed that piperin could lower fat levels and performed as an anti-obesity agent [10]. Adult male rats used in this study were Wistar rats aged 2.5-3 months weighing 200-250 grams. From the results of weight measurement (Table 1), it is known that rats in the *P. nigrum* 6.66 mg and *P. nigrum* 13.32 mg groups showed significant weight loss at the end of the week compared to other groups.

Frequency of Total Penile Reflex (TPR) in the TU, *P. nigrum* 3.33 mg, *P. nigrum* 6.66 mg and *P. nigrum 13.32* mg groups increased until 5th week observation and decreased in the next week observation. Penile erection as a result of sexual stimuli was transmitted from the central to peripheral, involving the activity of neurotransmitters such as dopamine and nitric oxide[11]. Flavonoids are known to play a role in modulating NO bioavailability through their action in regulating the expression of the enzyme nitric oxide synthase (NOS) [12]. Piperin also showed its role in improving bioavailability of NO. Previous study showed that piperine has the potency to protect nitric oxide from free radicals by increasing its bioavailability. In addition, there is an indication that piperine is also involved in enhancing the activity of endothelial nitric oxide (eNO) [5].

Physical factor associated with sexual desire and erection capability is the level of the hormone testosterone [13]. Piperin is known to have the ability to inhibit the action of 5αreductase enzymes so that testosterone is not converted to DHT and keeps the levels high [14]. Previous study declared that flavonoid were also shown to increase StAR gene expression in Leydig male mice cells. StAR protein involved in cholesterol transport was as a source of steroid hormone synthesis [6].

Decreased levels of the 8th week of testosterone in the *P. nigrum* 3.33 mg, *P. nigrum* 6.66 mg and *P. nigrum* 13.32 mg groups, could be due to elevated hormone levels at week 4 examination causing the negative feedback process on the hypothalamus-piyuitary-gonadal axis. Piperin had the same effect as enzyme inhibitor of 5αreductase. Therefore, testosterone levels remain high by not converted to DHT [14].

The decrease in erectile ability of the TU, *P. nigrum* 3.33 mg, *P. nigrum* 6.66 mg and *P. nigrum* 13.32 mg groups at 6th, 7th and 8th week observations was probably related to the decrease of testosterone levels at the 8th week. Low levels of the hormone testosterone will lead to decreased muscle relaxation abilities mediated by NO associated with decreased expression of NOS, smooth muscle cell apoptosis, increased extracellular matrix deposition and fat cell accumulation in the subtunica area of the corpus cavernosum, fibrosis in the tunica albuginea, failure of neural supply due to damage of the nerve structure in the dorsal portion of the penis, endothelial morphological disorders and decreased trabecular smooth muscle [15-18].

Hormone testosterone diffuses through the plasma membrane and binds to the androgen receptor to initiate the mechanism of action [19]. Circulating testosterone activates the androgen receptor (AR) and also serves as the source of estrogen in the brain. Local androgen receptor, such as at foreskin of preputium is more needed to increase stimuli for erection. The results of androgen receptor concentration examination in brain tissue showed no significant differences among treatment groups (Table 4). Based on the mean concentration, the Na-CMC group had the highest concentrations of androgen receptors compared to the other groups. The examination results of these androgen receptor concentrations were consistent with the results of serum testosterone levels. Levels of the hormone testosterone in the circulation is a component that regulates expression of androgen receptors. Previous study using male rats indicated that castration could decrease the expression of androgen receptors, whereas administering testosterone might improve expression of androgen receptors in brain tissue [20].

V. CONCLUSION

Extract of black pepper fruits had erotogenic effect on the increase of testosterone level of male Wistar rats.

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