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The Effect of Fruit Extracts of Black Pepper on the Fertility Potential of Male Albino Rats

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Abstract This study aimed to determine the effects of black pepper fruit extract on serum testosterone status and fertility potential in male subjects. Twenty seven healthy male mice, four months old, weighing between 25-30 g, were used as experimental animals and grouped into three. The first group was given pellets that do not contain black pepper extract as the control. The second and third groups, respectively were treated with pellets containing water extract and ethanolic extract. Pellets given ad libitum every day for 90 days. Serum testosterone measured by radioimmunoassay (RIA) techniques. Left testis of mice removed, weighed and dissected. Cauda epididymis was separated from testis and squeezed to determine epididymal sperm concentration, motility, and morphology. To determine the diameter of the seminiferous tubules, the number of spermatocytes and spermatids, testes were fixed in Bouin solution, processed and cut at a thickness of 5 um, stained with hematoxylin and eosin and examined under a light microscope at 400x magnification. When compared with control group, male mice treated with fruit extract of black pepper showed significant increase in the serum testosterone level, epididymal sperm concentration, spermatocyte counts, spermatid counts, and the weight of epididymis tubules. As the conclusion, fruit extract of black pepper has a positive effect on androgenic hormone level and fertility potential in male mice.

Keywords: *piper nigrum, black pepper, testosterone, fertility, male mice*

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1. Introduction

Study results on the biological benefits of the pepper plants families for medicinal purposes are still a maze. Relating to reproductive function, the effects of pepper plant extract both on human and animals seem to contradict each other. Reference [1], for instance, found albino male rats (*Rattus norvegicus*) treated with diherbal mixture of *Zanthoxylum leprieurii* and *Piper guineense* showed significant increase in libido parameters. Reference [2] 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. The result of our previous study, through mating test, has also proved that black pepper extract can improve sexual drive in male mice [3].

However, there were many studies that seemed to contradict the above findings that make the effects of pepper against reproductive function become uncertain. Reference [4] wrote that piperine could allegedly damage the epididymal environment and sperm function due to its ability to increase the level of reactive oxygen species by lowering the activity of antioxidant enzymes and sialic acid level in the epididymis. Furthermore, [5] found that male mice treated orally with black pepper powder significantly shows antispermatogenic and antifertility effects.

Such findings supported by many other reports, as reviewed by [6], that the herb plants belong to pepper family, such as *Piper longum* and *Piper nigrum*, has the potential to be used as a contraceptive material. These findings strengthened by [7] that piperine treatment caused a significant reduction in the weights of testes, regression in seminiferous tubules, damages of germ cells, and spermatogenic arrest. On the other hand, mice treated with *P. longum* showed a significant increase in the weight of testes, sperm number and motility [8]. Although such positive claims had not been widely supported by adequate data, these facts should be considered as a scientific challenge.

Supposing piperine from *Piper guineense [1]* or *Piper retrofractum* [2] and *Piper longum* [8] is the key factor responsible for any reproductive and sexual effects in laboratory animals, the same active substance from *Piper nigrum* (black pepper) can also, at least, reveals similar phenomenon.

In an attempt to find out whether black pepper should be seen as a useful drug or not in relation to male fertility as well as sexuality we had applied two kinds of fruit extracts of black pepper, water extracts and ethanolic extracts, in mice.

By using only one species of pepper, *Piper nigrum*, the maze effects of pepper plants, *Piper guineense*, *Piper retrofractum*, *Piper longum*, and *Piper nigrum* on male

reproductive function would be minimized. Furthermore, by using water and ethanol as the solvent, it would be known which type of extraction methods should be applied in extracting the fruit of black pepper the best for treating male reproductive problems.

2. Methods

2.1. Extract Preparation and Dosage

Black pepper fruits (*Piper nigrum* Linn.) were obtained from pepper farmer in the District of Lampung Utara, Lampung Province, Indonesia. The procedure of extract preparation and the dosage calculation in this study was similar to our previous study [3] as follows. Two extract types of black pepper, ethanolic extract and water extract, were prepared. All extraction steps initiated with grinding the dried 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. To make water 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. 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 which is 5 mg/kg body weight. Trikatu is a generic Ayurvedic medicine prepared by mixing fruit powder of black pepper (*Piper nigrum* Linn.), fruit powder of long pepper (*Piper longum* Linn.) and rhizomes powder of ginger (*Zingiber officinalis* Rosc.) in the ratio of 1:1:1 [9].

Given Trikatu consist of three species, two peppers and one ginger, so that the tolerated limit of piperine dose should be 3.33 mg/kg. 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 animals.

2.2. Animals and Experimental Design

By using completely randomized design 27 male Swiss albino mice, 4 months old and weighing 25-30 g from Lampung Veterinary Center, Indonesia, were used. The experiental mice divided into 3 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 water extract and ethanolic extract. The treatment was carried out for 90 days. During treatment both pellet and water were available *ad libitum*.

2.3. Hormone Assay

At the end of 90 days experiments, mice's blood was collected by cardiac puncture after the animals deeply anesthetized. For separating serum, blood was centrifuged at 3000 rpm for 15 minutes and stored in freezer at -20^{o}C until use. Serum testosterone was measured by radioimmunoassay (RIA) technique.

2.4. Sperm and Testis Histological Studies

Immediately after the blood is drawn, the testes of mice dissected for assessing: testis weight, epididymis weight, diameter of seminiferous tubule, spermatocyte count, spermatid count, spermatozoa concentration, sperm motility and normal morphology. Testes and epididymis removed and weighted using analytical-density digital balance with readability of 0.0001g. Semen in the epididymis was squeezed and diluted with physiological saline. Spermatozoa was counted using a Neubauer's haemocytometer under a light microscope at 400x magnification and expressed as million/ml of suspension. Quantitative epididymal sperm motility expressed as an index determined by counting both motile and immotile spermatozoa per unit area.

Sperm morphology was assessed from a smear of the epididymal filtrate prepared on a clean glass slides by addition of a drop of 1% eosin. After the object dried observation done under a light microscope at 400x magnification and abnormalities of either head or tail were noted. To assess the seminiferous tubular diameters and spermatocyte as well as spermatid count, left testis from each mouse was fixed in Bouin's solution. Transverse sections of the organs were cut at 5 µm and stained with hematoxylin and eosin and examined under a light microscope. The tubular diameter was determined by measuring the width of the tubules using occulometer. Spermatocytes and spermatids counts were done at 10 seminiferous tubules of each experimental unit and then averaged.

2.5. Statistical Analysis

The data, presented as Mean ± SEM, was analysed with a one-way ANOVA. Means were separated using Least Significance Difference (LSD) test. All of the statistics that applied are programmed in SPSS version 18.

3. Results

All effects of treatment on serum hormone, testicular histologic and sperm parameters are shown in Table 1. Serum testosterone of mice given water extract and ethanolic extract of black pepper fruits was significantly higher compared to that of control group ($p < 0.01$). The concentration of spermatozoa, the spermatocyte and spermatid counts of male mice fed water and ethanolic extracts were significantly higher compared to that of control. When the effects of aqueous and ethanolic extracts of black pepper were compared, ethanol extract showed a higher effect than that of water extract. On the other hand both water and ethanol extract of black pepper showed no significant effect on sperm motility and morphology.

Table 1. Effects of black pepper extract on serum testosterone and testicular parameters of mice

Parameters	Treatment			ANOVA	
	Control	Water Extract	Ethanol Extract	F	P
Serum testosterone (ng/ml)	0.863±0.084[a]	2.872±0.507[b]	2.967±0.276[b]	12.423	0.000
Spermatozoa concentration (million/ml)	9.322±0.755[a]	18.133±1.776[b]	26.578±2.270[c]	25.159	0.000
Normal morphology (%)	47.741±3.446[a]	53.213±3.474[a]	54.543±2.937[a]	1.197	0.319
Sperm motility (%)	43.889±3.310[a]	56.111±3.203[a]	51.667±3.005[a]	3.796	0.037
Testis weight (mg)	100.433±2.123[a]	101.467±1.208[a]	103.992±1.445[a]	1.196	0.320
Epididymis weight (mg)	22.700±0.676[a]	29.755±1.482[b]	30.167±0.987[b]	14.558	0.000
Diameter of seminiferous tubule (μm)	156.632±3.659[a]	148.828±16.820[a]	167.418±3.352[a]	0.850	0.440
Spermatocyte counts	11.268±0.410[a]	18.780±2.547[b]	27.132±1.947[c]	18.081	0.000
Spermatid counts	25.397±1.332[a]	44.183±1.771[b]	57.926±2.785[c]	63.165	0.000

Values are mean ± SE; values of each parameter that share the same superscript are not significantly different at α=0.05 using LSD test.

On the testicular parameters, fruit extract of black pepper has no effect on the testis weight and the diameter of seminiferous tubules but significantly increased the epididymis weight compared to that of control.

4. Discussion

High testosterone level in rats fed the extract of pepper that revealed in this study is consistent with the findings of [10], that piperine supplementation might increase the plasma testosterone level. There are several possible role of piperine in increasing testosterone levels in mice. Piperine the active constituent of *Piper nigrum* is known to have an inhibitory effect on testosterone 5α-reductase. The inhibition of this enzyme causes testosterone levels remain high [11]. Black pepper fruit, as shown by [12], is rich in patty acid such as auric acid, myristic acid and palmitic acid. Fatty acid, as summarized by [13] in a review article, significantly affect the secretion and metabolism of androgens.

The next possibility that makes black pepper extract affect androgens secretion in male subject is minerals such as magnesium (Mg) and zinc (Zn). As reported by [14], black pepper contains magnesium (Mg) in a significantly high concentration. A supplement containing of Mg-Zn significantly increase free testosterone levels in strength-trained, competitive athletes [15].

That black pepper is known to contain a variety of nutrients, including zinc, has been reported by many authors. This nutrient is responsible for numerous physiological processes in organisms [16,17]. Zinc supplementation can improve the anti-oxidative status and hormone levels in goats [18] and proved to increase serum levels of sex hormones including testosterone in rats [19].

Such zinc content was might explain the high number of spermatocytes, spermatids, and spermatozoa in mice fed water extract as well as ethanolic extract of black pepper fruit as shown in Table 1. In men, zinc administration make seminal zinc levels increased and significantly improve the sperm count, number of motile and normal spermatozoa [20]. Zinc, as considered by [21], is an essential trace element for the maintenance of germ cells, spermatogenesis development and regulation of sperm motility.

As can be seen in Table 1, mice treated with pepper extract did not show significant changes in the weight of testis and the diameter of seminiferous tubules but showed a significant increase in epididymis weight. With these facts it can be presumed that the *Piper nigrum* fruit extract has the potential to improve spermatogenesis. It may be associated with the increased of testosterone level. This may be associated with elevated levels of testosterone. This is consistent with the findings of previous studies that spermatogenesis is strongly associated with testosterone. [22,23,24].

This study results clearly showed that there was no significant effect of black pepper extracts on sperm motility. However there was a report that men with poor sperm motility showed greater improvement in response to zinc sulphate possible due to a synergistic effect of zinc and androgen [25]. Because sperm moitility depended uppon dihydrotestosterone (DHT) while DHT itself affected by testosterone 5α-reductase [26], these contradiction seem to indicate that both water and ethanolic extract did not contain enough testosterone 5α-reductase inhibitory to change the DHT level.

The last finding of this study was that ethanolic extract of black pepper fruits showed significant effects on spermatocyte and spermatid counts and epididymal sperm concentration when compared with that of water extract. These facts seem to confirm that ethanolic extracts of black pepper has a broader spectrum of effects due to contain much more active ingredient than that of water extract. Ethanolic extract of black pepper showed antibacterial activity against all test bacteria compared with that of water extract [27,28].

In mice, ethanol extract dosage of 100 mg/kg showed a higher protective effect against gastric ulcer (90.9%) than that of water extract (41.2%) [29]. In addition, ethanolic extract of pepper showed higher antioxidant activity than that of water extract [30,31].

5. Conclusion

In conclusion, fruit extract of black pepper, *Piper nigrum*, has a positive effect on testosterone level and fertility potential in male mice.

Confict of Interest

The Authors declare that there is no conflict of interest.

References

[1] Kpomah E.D., Uwakwe A.A., Abbey B.W., "Aphrodisiac studies of diherbal mixture of *Zanthoxylum leprieurii* Guill. And Perr. and

Piper guineense Schumach. and Thonn. on male wistar rats," Global J. Res. Med. Plants Indigen. Med. 1, 381-390. Sep. 2012.

[2] Rahmawati N. and Bachri M.S., "The aphrodisiac effect and toxicity of combination *Piper retrofractum* L, *Centella asiatica* and *Curcuma domestica* infusion," Health Sci. J. Indonesia. 3, 19-22. Jun.2012.

[3] Sutyarso, Kanedi M., Rosa E., "Effects of black pepper (*Piper nigrum* Linn.) extract on sexual drive in male mice," Research Journal of Medicinal Plant 9 (1): 42-47., 2015.

[4] D'Cruz S.C. and Mathur P.P., "Effect of piperine on the epididymis of adult male rats," Asian J. Androl. 7, 363-368. Dec.2005.

[5] Dalal, P. K. Tripathi,A and Gupta, S. K.., "Vajikarana: Treatment of sexual dysfunctions based on Indian concepts," Indian J Psychiatry. 55 (Suppl 2): S273-S276. Jan. 2013.

[6] Ogbuewu I.P., Unamba-Oparah I.C., Odoemenam V.U., Etuk I.F., Okoli I.C., "The potentiality of medicinal plants as the source of new contraceptive principles in males," North Am. J. Med Sci. 3, 255-263. Jun. 2011.

[7] Ukoha U.U., Egwu O.A., Dimkpa U., Maduka S.O., Okafor J.I., Ndukwe G.U., Ogonnadi K., "Histological and weight changes in testes of male albino rats fed with diets containing yaji (A Local Meat Sauce)," Int. J. Health Sci. Res. 4 (8), 118-126. 2014.

[8] Mohammadi F., Nikzad H., Taherian A., Mahabadi J.A., Salehi M., "Effects of herbal medicine on male infertility," Anatomical Science. 10(4), 3-15. 2013.

[9] Chanda D., Shanker K., Pal A., Luqman S., Bawankule D.U., Mani D., Darokar M.P., "Safety evaluation of Trikatu, a generic ayurvedic medicine in charles foster rats," J. Toxicol. Sci. 34, 99-108. 2009.

[10] Vijayakumar R.S. and Nalini N., "Piperine, an active principle from *Piper nigrum*, modulates hormonal and apolipoprotein profiles in hyperlipidemic rats," J. Basic Clin. Physiol. Pharmacol.17, 71-86. 2006.

[11] Hirata N., Tokunaga M., Naruto S., Iinuma M., Matsuda H., "Testosterone 5α-reductase inhibitory active constituents of *Piper nigrum* leaf," Biol. Pharm. Bull. 30, 2402-2405. Aug./ 2007.

[12] Meghwal M. and Goswami T.K., "Chemical composition, nutritional, medicinal and functional properties of black pepper: A review," Open Access Scient. Rep. Vol.1. 2012.

[13] Gromadzka-Ostrowska J., "Effects of dietary fat on androgen secretion and metabolism," Reproduct. Biol. 6 Suppl 2:13-20. 2006.

[14] Bouba A.A., Njintang N.Y., Foyet H.S., Scher J., Montet, D., Mbofung C.M.F., "Proximate composition, mineral and vitamin content of some wild plants used as spices in Cameroon," Food Nutr. Sci. 3, 423-432. Jul.2012.

[15] Brilla L.R. and Conte V., "Effects of a novel zinc-magnesium formulation on hormones and strength," J. Exercise Physiologyonline. 3, 26-36. Oct. 2000.

[16] Nkansah M.A. and Amoako C.O., "Heavy metal content of some common spices available in markets in the Kumasi metropolis of Ghana," Am. J. Sci. Ind. Res. 1, 158-163. 2010.

[17] Hamza S., Srinivasan V., Dinesh R., "Nutrient diagnosis of black pepper (Piper nigrum L.) gardens in Kerala and Karnataka," J. Spices Arom. Crops. 16, 77-81. Sep.2007.

[18] Kumar P., Yadav B., Yadav S., "Effect of zinc and selenium supplementation on antioxidative status of seminal plasma and testosterone, T and T level in goat blood serum," J. Applied Anim. Res. 41, 382-386. May. 2013.

[19] Egwurugwu J.N., Ifedi C.U., Uchefuna R.C., Ezeokafor E.N., Alagwu E.A., "Effects of zinc on male sex hormones and semen quality in rats," Niger. J. Physiol. Sci. 28, 17-22. Jun.2013.

[20] Tikkiwal M., Ajmera R.L., Mathur N.K., "Effect of zinc administration on seminal zinc and fertility of oligospermic males," Indian J Physiol Pharmacol. 31(1), 30-4. Jan-Mar.1987.

[21] Yamaguchia, S., Miura, C., Kikuchi K.,, Fritzie T. Celino, F.T., Tetsuro Agusa T., Tanabe S.and Miuraa, T., "Zinc is an essential trace element for spermatogenesis" PNAS 106(26): 10859-10864. Jun. 2009.

[22] McLachlan R.I., Wreford N.G., Meachem S.J., de Kretser D.M., Robertson D.M., "Effects of testosterone on spermatogenic cell populations in the adult rat," Biology of Reproduction. 51, 945-955.Jun. 1994.

[23] Nusier M.K, Bataineh H.N., Bataineh Z.M., Daradka H.M., "Effect of frankincense (*Boswellia thurifera*) on reproductive system in adult male rat," Journal of Health Science. 53(4), 365-370. May.2007.

[24] Ruwanpura S.M, McLachlan R.I., Meachem S.J., "Hormonal regulation of male germ cell development,". Journal of Endocrinology. 205, 117-131. Feb.2010.

[25] Takihara H, M.J. Cosentino, and A.T. Cockett., "Effect of low-dose androgen and zinc sulfate on sperm motility and seminal zinc levels in infertile men," Urology, 22(2):160-4. Aug.1983.

[26] Henderson N.A., Cooke G.M., Robaire B., "Region-specific expression of androgen and growth factor pathway genes in the rat epididymis and the effects of dual 5α-reductase inhibition," Journal of Endocrinology. 190, 779-791. 2006. Link

[27] Pundir R.K and Jain P., "Comparative studies on the antimicrobial activity of black pepper (*Piper Nigrum*) and turmeric (*Curcuma Longa*) extracts," IJABPT. 1(2), 491-501. Aug 2010.

[28] Tahir, A. and Moeen, R., "Comparison of antibacterial activity of water and ethanol extracts of Camellia sinensis (L.) Kuntze against dental caries and detection of antibacterial components," Journal of Medicinal Plants Research Vol. 5(18), pp. 4504-4510, Sep. 2011.

[29] Bae, J.Y., Lee, Y.S., Han, S.Y., Jeong, E.J., Lee, M.K., Kong, J.Y., Lee, D.H., Cho, K.J., Lee, H.S. and Ahn, M.J., "A Comparison between Water and Ethanol Extracts of Rumex acetosa for Protective Effects on Gastric Ulcers in Mice" Biomol. Ther. (Seoul), 20(4): 425-430. Jul. 2012.

[30] Nahak G. and Sahu R.K., Phytochemical evaluation and antioxidant activity of *Piper cubeba* and *Piper nigrum*. J. Applied Pharmaceutical Sci. 01 (08), 153-157. Oct. 2011.

[31] Hakkim, F.L., Girija, S., Kumar, R.S. and Jalaludeen, M.D., "Effect of aqueous and ethanol extracts of Cassia auriculata L. flowers on diabetes using alloxan induced diabetic rats" Int J Diabetes & Metabolism 15: 100-106. Jan.2007.