EFFECT OF PURWOCENG EXTRACT ON THE EXPRESSION OF NITRIC OXIDE NEURONAL SYNTHASE IN PENILE TISSUE OF WHITE MALE RATTUS NOVERGICUS

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ABSTRACT

Objective: *To know the effect of purwoceng extract (Pimpinella alpine) on the expression of neuronal nitric oxide synthase (nNOS) in the penile tissue of white male rats (Rattus novergicus) through immunohistochemical examination.* ***Material & Methods:*** *Animal experiments consisted of 27 male rats, three months of age with a mean body weight of approximately 200 grams were divided into three groups randomly, one group consisted of 10 rats. Treatment 1 rats receive Pimpinella alpina extract with 50 mg/ml, Treatment 2 rats receive Pimpinella alpina extract with 100 mg/ml. In the control group rats receive aquadest as much as 1 ml.* ***Results:*** *In the control group the average is 75.67, in the treatment group 1 is 113, and in group 2 is 181.44. The difference in treatment between groups was performed using one-way ANOVA test. Between control and treatment groups 1 the different is significant with p value of 0.013 ($p<0.05$). The mean expression of nNOS between treatment groups 1 and 2 differ significantly with $p=0.00$ ($p<0.05$).* ***Conclusion:*** *Purwoceng extract (Pimpinella alpine) can increase the expression of nNOS on NANC nerve fibers (nonadrenergic noncholinergic) in the corpora cavernosa of the penis that causes smooth muscle relaxation in penile erection.*

Keywords: *Purwoceng (Pimpinella alpine), neuronal nitric oxide synthase (nNOS), erection.*

ABSTRAK

Tujuan: *Mengetahui sejauh mana pemberian ekstrak purwoceng (Pimpinella alpine) berpengaruh terhadap ekspresi neuronal nitric oxide synthase (nNOS) pada penis tikus putih jantan melalui pemeriksaan imunohistokimia.* ***Bahan & cara:*** *Hewan percobaan terdiri dari 27 ekor tikus putih jantan, usia tiga bulan dengan rerata berat badan 200 gram dibagi menjadi tiga kelompok secara acak, 1 kelompok terdiri dari 10 tikus dilakukan 5 hari adaptasi di kandang kemudian diberi perlakuan sebagai berikut: perlakuan 1 diberi larutan ekstrak Pimpinella alpina sebanyak 50 mg/ml, perlakuan 2 diberi larutan ekstrak Pimpinella alpine sebanyak 100 mg/ml, pada kelompok kontrol diberi aquadest sebanyak 1 ml.* ***Hasil:*** *Pada kelompok kontrol didapatkan rerata sebesar 75.67, pada kelompok perlakuan 1 sebesar 113, dan pada kelompok 2 sebesar 181.44. Perbedaan perlakuan antar kelompok dilakukan dengan menggunakan tes one way anova. Antara kelompok kontrol dan perlakuan 1 berbeda secara signifikan dengan $p=0.013$ ($p<0.05$). Rerata ekspresi nNOS antara kelompok perlakuan 1 dan 2 berbeda secara signifikan dengan $p=0.00$ ($p<0.05$).* ***Simpulan:*** *Ekstrak purwoceng (Pimpinella alpine) dapat meningkatkan ekspresi nNOS pada serabut saraf NANC (nonadrenergic noncholinergic) di corpora cavernosa penis yang menyebabkan relaksasi otot polos yang menyebabkan ereksi penis.*

Kata kunci: *Purwoceng (Pimpinella alpine), neuronal nitric oxide synthase (nNOS), ereksi.*

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INTRODUCTION

Purwoceng is a commercial herbal plant whose roots are reported to be efficacious as an aphrodisiac (increases sexual arousal and cause an erection), diuretics (diuretic), and tonic (able to increase stamina). This plant is native to Indonesia, grow in endemic places in mountainous regions like Dieng, Mount Pangrango (West Java). Currently purwoceng population is endangered because of a genetic erosion of large−scale. The purwoceng plants are found only in the Dieng plateau, cultivated in the isolated area in the village of Sekunang.[1]

Suzery isolate active compounds from plants found on purwoceng and stigmasterol were found, stigmasterol is a class of steroid saponin OH

groups attached to third carbon atom of siklopentano-perhidrofenantren core, so it can bond with oligo-saccharides. Steroid saponin soluble in water due to the glycoside bond form.[2] Otih reported that purwoceng has compound of alkaloids, glycosides, coumarin, triterpenoids-steroid, and saponin.[3]

Some researchers have examined the effect of purwoceng's root in mice. One technique used by Caropeboka is by castrating male mice and injected him with purwoceng root extract in olive oil (dose 20-40 mg). The observed effect is an increase in the prostate gland and seminal gland significantly compared with controls. These facts give an indication of androgenic activity of the extract of the roots purwoceng. Conversely, when the female rats without ovaries injected with purwoceng root extract in olive oil at the same dosage, there is an increase in weight of the uterus. These results showed estrogenic activity of the purwoceng roots extract. Another test was done on male chicks that showed androgenic effects of purwoceng root extract at a dose of 30% which is characterized by an increase in the size of the comb and increased weight of the testis.[2] The research was supported by the results from the research of Taufiqqurrachman reported that purwoceng root extract as much as 50 mg can increase levels of the hormone LH (luteinizing hormone) and testosterone compared with controls (without extract) in Sprague Dawley rats. Interestingly, the effect purwoceng was also compared with the effects of other natural ingredients such as earth peg extract. The results showed that at a dose of 25 mg, Earth peg extract has the effect of increasing the LH higher than purwoceng, but on the contrary if the dose was increased to 50 mg, at a dose of 50 mg, purwoceng also gives the effect of increasing the LH higher than the earth peg extract. But when purwoceng extract mixed with earth pegs at the same dosage (each extract with 25 mg), the effect of increasing level of testosterone is higher than other treatment.[4]

Nasihun conducted research (2009) with Sprague Dawley rats, purwoceng extract (Pimpinella alpine Molk) has an influence on increasing vitality as the indicator is the elevated level of testosterone and luteinizing hormone, but did not increase the levels of FSH.[5]

In Suhartinah study, purwoceng extract was given to Wistar rats and the rats that received Purwoceng extract have sperm count and motility better than the control. The weight of the testes of rats that received purwoceng extract were heavier when compared to control.[6]

Juniarto reported that purwoceng root extracts given on Spraque Dawley rats may also increase the degree of spermatogenesis in the testes, the number and motility compared with controls (without purwoceng), but did not differ with the earth peg extract.[7]

Currently many herbal products containing purwoceng extract are circulating in pharmaceutical store. Some of these herbal products containing purwoceng extract (Pimpinella alpina) in the absence of scientific evidence to improve erections in men. These herbal drugs were still commercialized, but many users complain of complications with the use of the drugs. Therefore the Food and Drug Monitoring Agency (BPOM) issued a decision to withdraw the drugs from circulation.

Relaxation of erectile tissue on corpus caver-nosum need nitric oxide from nonadrenergic-non-cholinergic and endothelial neuron. Penile tissue of diabetic mellitus patient showed disturbance in smooth muscle relaxation which is mediated by neurogenic factor and endothelial, the increase of advanced glycation end products (AGEs), and upregulation of arginase which is competitor of nitric oxide synthase (NOS) with the substrate, L-arginin, therefore there will be a decrease in the synthesis, transmission and activation of nitric oxide. As a result of diabetes mellitus, there will be progressive loss of normal smooth muscle and endothelial from the corpus cavernosum which will be replaced by fibrotic tissue which later cause complete erectile dysfunction.[8-10]

Neuronal nitric oxide synthase (nNOS) is one of the major NOS isoform responsible for the synthesis of physiological mediator of penile erection. Stimulation of the postsynaptic nerve terminals nNOS on nonadrenergic−noncholinergic emerged as a process that leads to penile erection. Neuronal nitric oxide synthase (nNOS) activation occurs via ionic calciu/calmodulin in place of the enzyme. This response can be inhibited by stimulation by binding factors−additional factors such as inhibitor protein of NOS (PIN), the NMDA receptor, or ligand carboxy−terminal PDZ on nNOS (CAPON).[11]

In the study conducted by Cashen et al., nNOS has great influence on the erection of the penis through the induction of rats using L-NA methylester (L-NAME).[12] According to Shabsigh et al, testosterone affects the penile erection through the

induction of nNOS. In rats receiving radiation of the prostate on 1000−2000 cGy, histologic evaluation showed a decrease in nerve fibers containing nNOS.[13]

Androgens particularly testosterone, are important for the maintenance of male sexual function.[14,15] Several research showed that testosterone and its metabolites increase sexual desire and sexual activity.[16] Other studies have shown that testosterone regulate penile erection by regulating nitric oxide synthase in the peripheral nervous system.[17,18] A decline in androgen could decrease smooth muscle cells of the corpora cavernosa through apoptosis process.[19] These findings suggested a link to the androgen physiology of erection.

OBJECTIVE

To know the effect of purwoceng extract (Pimpinella alpine) on the expression of neuronal nitric oxide synthase (nNOS) in the penile tissue of white male rats (Rattus novergicus) through immuno-histochemical examination.

MATERIAL & METHODS

The research conducted is a purely experimental research in which the treatment group were given purwoceng (Pimpinela alpine). Research design using a design of Randomized Experimental Post Test Only Control Group Design.

Animal experiments consisted of 27 male rats, three months of age with a mean body weight of approximately 200 grams were divided into three groups randomly, one group consisted of 10 rats. All rats will go through 5 days of adaptation in the Integrated Research and Testing Laboratory (LPPT) Gadjah Mada University, the groups are as follows: Treatment 1 rats receive Pimpinella alpina extract with 50 mg/ml. Treatment 2 rats receive Pimpinella alpina extract with 100 mg/ml. In the control group rats receive aquadest as much as 1 ml.

All administration is done every 7 am during 9 consecutive days - respectively. On the 10th day prior to the removal of tissue, male rats were exposed to female rats in a box which is sealed with a wire gauze for 10-15 minutes to achieve an erection. Then male rats were sacrifice with anesthetic by using Ketamine, retrieved penile tissue from rats and place into paraformaldehide 4% (PFA) for examination nNOS expression through immunohistochemistry examination.

RESULTS

In the control group the average is 75.67, in the treatment group 1 is 113, and in group 2 is 181.44. The difference in treatment between groups was performed using one-way ANOVA test. Between control and treatment groups 1 the different is significant with p value of 0.013 ($p<0.05$). The mean expression of nNOS between treatment groups 1 and 2 differ significantly with $p=0.00$ ($p<0.05$).

Table 1. Frequency of nNOS expression in the nucleus of NANC nerve cell in corpora cavernosa with the visual field of two cavernosa and magnification of 400 times (the result can be seen in the Appendix).

Control	P 1	P 2
58	157	191
81	112	232
78	110	219
105	139	161
100	59	157
73	120	172
64	137	168
72	99	176
50	84	157

Here is an overview of histopathology immunohistochemistry results on each group with a magnification of 400 times.

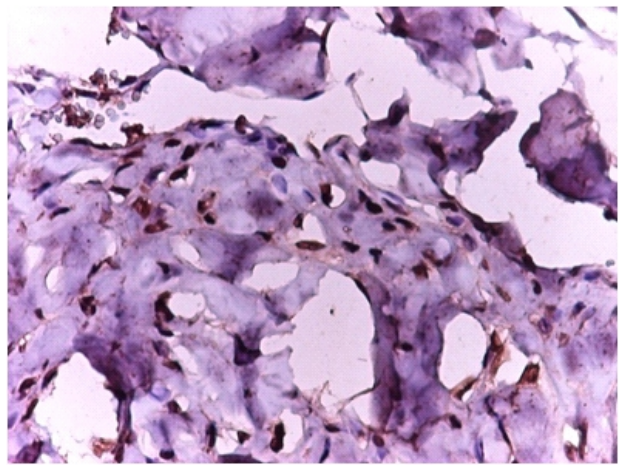

Figure 1. Immunohistology nNOS expression in the corpora cavernosa control group.

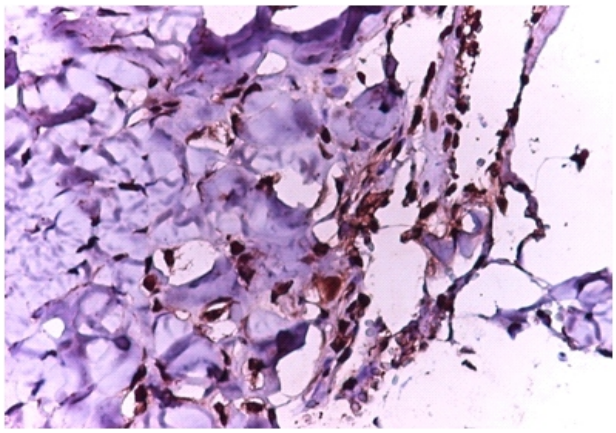

Figure 2. Immunohistochemical expression of nNOS in the corpora cavernosa treatment group 1.

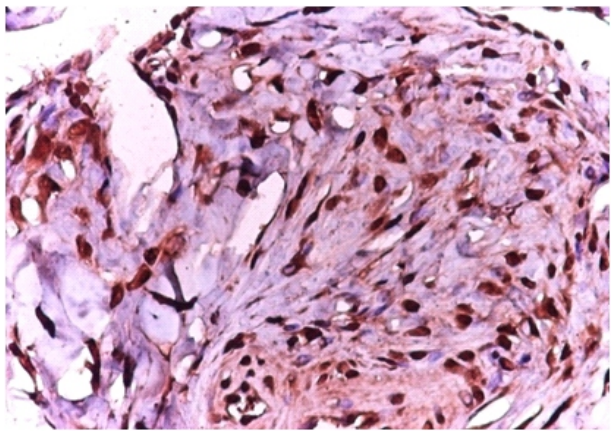

Figure 3. Immunohistochemical expression of nNOS in the corpora cavernosa treatment group 2.

DISCUSSION

Corpora cavernosa is a hollow network composed of trabecular muscle - smooth muscle and interconnected. Septum between the two corpora cavernosa containing many holes that allow the flow of blood from the corpus cavernosa into the corpus cavernosa one another, so that essentially the two corpora cavernosa is a unitary unit. Corpora cavernosa in young men is composed of 40-52% of smooth muscle, in older man with the corporal veno occlusive dysfunction (CVOD) consists of 19-36% of smooth muscle, and in men with erectile dysfunction containing 10-25% smooth muscle with increase in collagen.

Tunica corpora cavernosa is a bilayer structure with multiple sublayer. The inner layer consists of a network of hollow and arranged circularly. The outer layer is composed of longitudinal which determine the thickness and strength of the tunica. While the tunica of the corpus spongiosum has no outer layer, so that the pressure is lower at the time of erection and prevent compression of the urethra during erection.

Arteri that supply the cavernosal space in the corpus cavernosum is artery penis profundus and branches of artery dorsalis penis which penetrate tunica albuginea along dorsum penis, particularly near gland of penis. At the site of the entrance, these arteries are divided into branches at trabecula and branches that are curvaceous are called the arteriole helicinae, which end into cavernosal space. Arteriole helicinae are plentiful at posterior area of corpus cavernosum. Corpus spongiosum received blood supply from artery bulbi urethrae and artery urethralis.[20]

The blood flow penile vein are complex compare to artery. Blood flow from the three corpus originate from venula which are near tunica albuginea. These venula formed emisari vein which exit from tunica albuginea then enter one of the four system of main penile vein; Vena dorsalis superficialis, Vena dorsalis profundus, Vena cavernosa and. Vena urethra. Deybach et al. state that Vena cavernosa are main vein at corpus cavernosum through Vena dorsalis profundus. The return of blood accumulate at Vena dorsalis penis profundus and end at plexus prostaticus.[21] The innervation of penis are divided into two parts, otonomic nervous system and somatic nervous system. Otonomic nervous system consist of parasympathetic nervous system which is from the S2-S4 segmen and sympathetic nervous system which is from the T10-L2 segment. Somatic nervous system worked through nervus-pudendalis. Sympathetic nervous system reached corpora, prostate and vesical urinary through nervus-hypo-gastricus.

Parasympathetic nervous system originate from sacral erection center and cell body which are located in intermediolateral nuclei S2-S4. After it exit from foramina sacralis, these nerves heading towards rectum as nervuserigentes to reach plexus pelvicus. In this location, preganglionic fibers inside ganglion and postganglionic nonadrenergic non cholinergic (NANC) fibers through nervus-cavernosus inside corpus cavernosum.[22]

Nitric oxide (NO) is found in the pelvic plexus, the cavernous nerve, and the nerve terminals of the corpora cavernosa, a branch of the dorsal

penile nerve, and the nerve plexus near the deep cavernous artery. NO is a gaseous substance released by endothelial cells that cause vasodilation due to the relaxation of vascular smooth muscle. NO acts as a paracrine on target cells. In the body, the half-life of NO is only a few seconds. NO synthesis in the body requires NO synthase (NOS), which has three main isoforms: neuronal NOS (nNos or NOS I), inducible NOS (iNOS or NOS II), and endothelial NOS (eNOS or NOS III).[23]

At the same nerves there are NADPH (nicotinamide adenine dinukleotide phosphate diaphorase), obtained from the activity of nitric oxide synthase (NOS) in neurons.Research conducted by David et al., Stated that penile erections in rats depends entirely on androgens for androgen regulate the function of NOS activity in the penis and other factors.[24]

Research conducted by Raquel et al., there is a significant decline in nNOS and eNOS on rats post castration. But when given testosterone, erectile function return because nitric oxide have response towards cavernosal tissue which positively correlate with level of nNOS and eNOS. Both isoform have large influence on erectile function.[25]

Currently very few research that study about the benefit of Pimpinella alpine on the increase of erectile function. The research are still very limited and sadly many herbal drug companies are commercializing the Purwoceng extract. Further research need to be done in order to determine the potential of the purwoceng extract.

In Nasihun study (2009), Purwoceng extract have influence on the increase vitality as a result of the increase of testosterone and luteinizing hormone but did not increase FSH.[5] With the increase of testosterone there will be an increase on expression of nNOS. In this study, there were an increase number of cell nucleus that were stained with antibody of nNOS in group treatment two. Those cells that were stained are the neuron NANC which secrete NO that relax the smooth muscle on the corpora cavernosa of the penis.

Several other studies abroad related to herbs and their use in erectile dysfunction include Epimedium wanshanense containing an active substance Icariin. In the study conducted by Wu-Jiang et al, groups of rats that were performed castration compared with normal rats. On examination of smooth muscle tissue, the levels of neuronal nitric oxide synthase and inducible nitric oxide synthase increased in the group of rats that were performed castration.[26]

CONCLUSION

Purwoceng extract (Pimpinella alpine) can increase the expression of nNOS on NANC nerve fibers (nonadrenergic noncholinergic) in the corpora cavernosa of the penis that causes smooth muscle relaxation in penile erection.

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