A PRELIMINARY STUDY OF APHRODISIAC PROPERTY FROM PORCUPINE TAIL MEAT ETHANOL EXTRACT IN MALE MICE

STUDI AWAL MENGENAI KHASIAT AFRODISIAK DARI EKSTRAK ETANOL DAGING EKOR LANDAK PADA MENCIT JANTAN

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ABSTRAK

Penelitian ini bertujuan untuk mempelajari khasiat afrodisiak ekstrak etanol dari daging ekor landak (*Hystrix javanica* F. Cuvier, 1823) yang oleh masyarakat di Jawa (Indonesia) diyakini dapat meningkatkan vitalitas dan kinerja seksual pria. Satu jam setelah pemberian ekstrak dan bahan kontrol, dua puluh mencit jantan yang belum berpengalaman secara seksualdibagi secara acak ke dalam empat kelompok dan dipasangkan dengan mencit betina. Mencit jantan diberikan ekstrak etanol dalam dua dosis; 150 dan 750 mg/kg BB (*p.o*). Sebagai kontrol positif digunakan sildenafil citrate 5 mg/kg dan 5% Tween 80 sebagai control negatif. Selama pengamatan terlihat adanya parameter perilaku seksual seperti *mounting* dan intromisi. Mencit jantan yang diberi ekstrak etanol pada dosis 750 mg/kg menunjukkan frekuensi *mounting* dan *intromission* yang lebih tinggi dibandingkan ekstrak etanol 150 mg/kg dan setelah 2 jam kelompok ini menunjukkan frekuensi yang tertinggi. Namun, ekstrak etanol pada kedua dosis tersebut belum dapat menurunkan waktu latensi *mounting* dan *intromission* serendah Kontrol positif.Temuan ini merupakan bukti awal mengenai khasiat afrodisiak ekstrak etanol daging ekor landak.

Kata kunci: Ekstrak daging, frekuensi intromisi, frekuensi mounting, potensi afrodisiak, stimulan seksual

ABSTRACT

This study aim to examine the aphrodisiac activity of ethanol extract from the tail meat of porcupine (*Hystrix javanica* F. Cuvier, 1823) that is traditionally believed by people in Java (Indonesia) could enhance male vitality and sexual performance. Twenty sexually inexperienced male mice were randomly divided into four groups and paired with artificially estrus female mice after one hour of drug an extract administration. Two doses of ethanol extract; 150 and 750 mg/kg were administered (*p.o*) to male mice. Sildenafil citrate was used as the positive control while 5% Tween 80 solution used as the negative control. Sexual behavior parameters such as mounting and intromission were observed for three hours of mating. Male mice treated with ethanol extract of porcupine tail meat at the dose 750 mg/kg BW showed higher mounting and intromission frequency compared to the group of ethanol extract dose 150 mg/kg. After 2 hours of administration showed the highest frequency compared to all groups. However, the ethanol extract could not reduce the mounting and intromission latency as low as sildenafil citrated treated group. Present findings provide preliminary evidence of aphrodisiac properties from the ethanol extract of porcupine tail meat.

Keywords: Aphrodisiac potency, intromission frequency, meat extract, mounting frequency, sexual stimulant

INTRODUCTION

Sexual function has a significant impact in determining the overall quality of life and individual well-being (Wagner *et al.* 2000). The sexual problem also links with socioeconomic development since according to WHO majority of the urban population in developed and developing countries is suffering from sexual dysfunctions (Asuntha *et al.* 2014). Therefore treatment to overcome the sexual problem that mainly related with sexual desire and male erectile dysfunction is needed.

Aphrodisiac substances are often used to enhance sexual performance and treat sexu-

al dysfunction. The modern definition of aphrodisiac can vary, but it is generally regarded as a substance that increases sexual desire (i.e. libido) and/or sexual pleasure (Melnyk & Marcone 2011) and it include those substances which aid in the proper functioning of the male and female sex organs (Sandroni 2001; Shamloul 2010). The substances can vary from foods, beverages, vitamins, minerals to other natural or synthetic chemicals (Krychman *et al*. 2007).

There are varieties of foods and drinks that were claimed by diverse cultures could improve men stamina, libido, and sexual function. Aphrodisiac found widely in various plant and animal sources and even though were not yet scientifically confirmed, many cultures practice that knowledge to treat those with asexual problem. Several meat sources that are believed by different cultures as aphrodisiacsare offal, bulltesticles (in Spain), lambeyes (in the Balkans), rooster'scombs (in Hungary), shrimps, lobsters, octopuses, oyster, fish and caviar (Migdal & Zivkovic 2007). Other animals from marine resources that have been studied due to having an indication of aphrodisiac values are sea cucumbers, oysters and sea slug (Nurjanah *et al*. 2009; Rizwan *et al*. 2013; Hashim et *al*. 2014).

One of the natural sources that were believed by people in Java (Indonesia) could improve men stamina and libido are porcupine tail meat (Prasetya 2012). The meat from porcupine tail was cooked with daily food recipe and eaten to gain the aphrodisiac effect. The reason of why the aphrodisiac effect is gained from the tail meat and not from the carcass is still unclear. However, our previous study showed that the tail meat contains more protein than the carcass (Anita *et al*. 2014). To the best of our knowledge, there were no scientific studies has been conducted regarding the aphrodisiac property of porcupine tail meat. This custom could risk the number of porcupine in the environment. However, if the benefit could be confirmed scientifically then it could open the potency for further utilization. Therefore, this research was conducted to study the aphrodisiac properties of ethanol extract of the porcupine tail meat in male mice.

RESEARCH METHODS

Porcupine (*Hystrix javanica* F. Cuvier, 1823) meat tails were obtained from the breeding results of Java porcupine in a captive breeding facility in Zoology Division, Research Center for Biology, Indonesian Institute of Sciences. The tail was thawed then the spines, skin and bones were removed. The meat was collected and blended using an electrical blender. The delicate meat was then freeze-dried. The dried meat obtained was extracted with 95% ethanol using Soxhlet extraction (Soxhlet 1879). The extract then was dried under reduced pressure.

Sildenafil citrate was obtained from a local pharmacy. The ethanol extract and sildenafil citrate were suspended in distilled water using Tween 80 (5%) for oral administration. Ethinyloestradiol and progesterone were used to artificially bring female mice to estrus phase according to Pande and Pathak (2009) with modification and it was dissolved in corn oil for subcutaneous injection. All the drug solutions were prepared just before administration.

Six to eight weeks male and female mice (*Mus musculus*) of Deutsch DenkenYokken (DDY) strain were used for the study. The male mice weighing 45-55 g meanwhile the female were 30-40 g. All animals were housed in standard propylene cages and maintained under standard laboratory conditions. They were conditioned with the temperature at 25 – 26 °C and 12 h light/12 h dark cycle along with free access to local mice pellets diet and drinking water (*ad libitum)*. The animals were acclimatized to the laboratory conditions for 7 days before commencing the experiment.

All male mice were randomly grouped into 4 groups (n=5). They were placed individually in separate propylene cages during the experiment. The negative control group received only 5% Tween 80 and the positive control group received a suspension of sildenafil citrate at the dose of 5 mg/kg BW (*p.o*). The EE150 group received ethanol extracts of porcupine tail meat at the dose of 150 mg/kg (*p.o*) and EE750 was the group that received 750 mg/kg (*p.o*). The male mice were fasted two hours before drug and extract administration. The female mice were artificially brought to oestrus according to Pande and Pathak (2009) method with some modification by subcutaneous injection of ethinyl oestradiol (0.0056 mg/40 g) 48 hours before mating and progesterone (0.28 mg/40 g) 4 hours before mating. An hour after administration of drug and extracts to male mice, female mice were introduced into the cage. Male and female mice were remaining paired for 3 hours and experiments were performed during the dark cycle.

The following male sexual behavioral parameters were recorded: time from the introduction of female into the cage of the male up to the first mount or mounting latency (ML), number of mounts without intromission (MF), time from the introduction of the female up to the first intromission by the male or intromission latency (IL), number of intromission (IF). Mount was operationally defined as the male assuming the copulatory position but failing to achieve intromission characterized by lifting of the male's forebody over the hindquarter of the female and clasping her flanks with his forepaw. Intromission was defined as the male's penis entering the female's vagina.

Data were expressed as the mean of five replicates ± SEM. Means were analyzed using a one-way analysis of variance (ANOVA) with posthoc test and complemented with independent sample t-test. All the statistical analyses were done using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). P value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Throughout the duration of the experiment precopulatory activities were observed like chasing, anogenital sniffing and nosing. The observed precopulatory activities were an indication of sexual arousal. Mounting and intromission behavior was also observed and it was useful cues of vitality, libido and sexual performance. While the number of MF reflects sexual motivation or male's desire to seek out and approach a female for the purpose of mating, increase in the number of IF shows the efficiency of erection, penile orientation and the ease by which ejaculatory reflexes are acti-

Table 1. Mounting and intromission frequency average number of male mice in all group (n =5 ± SEM)

Group	Substance and Dose	Frequency					
		Mounting			Intromission		
		1st hour	2nd hour	3rd hour	1st hour	2nd hour	3rd hour
Negative	5 % Tween 80	3.80 ± 2.15	3.40 ± 2.18	3.60 ± 2.16	3.20 ± 2.72	2.60 ± 1.47	3.00 ± 2.14
Positive	Sildenafil citrate 5 mg/kg	8.80 ± 3.74	2.80 ± 1.50	2.40 ± 1.16	4.40 ± 3.29	2.40 ± 2.40	3.40 ± 2.36
EE150	Ethanol Extract 150 mg/kg	0.60 ± 0.60	0.00 ± 0.00	1.40 ± 0.87	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
EE750	Ethanol Extract 750 mg/kg	2.80 ± 1.74	4.80 ± 3.09	6.80 ± 2.52	0.80 ± 0.80	4.60 ± 3.64	10.00 ± 7.19

vated (Agmo 1997). Sexual motivation or desire is essential for initiation of sex and this is showed by ML where more motivation resulted in faster mounting.

Statistical analysis (results were not shown here) indicated that there were no significant differences between the extract groups and control groups. One possibility that caused this insignificant calculation is the condition of male mice. The male mice used in this study which was sexually inexperienced could also evoke high variances of respond to female presence. Sexual experience is an important modifier of male mating behavior. Initiation of sexual behavior in male rodents is triggered by the detection and investigation of female odor cues and male behavioral responses to female odors are sensitive to sexual experience (Swaney*et al*, 2012). Even though the statistical analysis showed insignificant differences between the extract and the control, the trends found from the experiment are interesting to discuss.

The average number of MF and IF of male mice in all groups are displayed in Table 1. An hour after drug administration male mice in the positive control group was very active in approaching and chasing the female compare to mice in other group resulted in the highest number of mountand intromission. However, the frequency decreased during the remaining time of the experiment. Meanwhile, in the negative control group, mounting and intromission were observed and the number was rather stable in the whole time of the experiment. EE150 group had the lowest MF and furthermore intromission behavior was not observed at all. The MF and IF of EE750 group increased overtime resulting in the highest number at the end of the experiment. The high IF in EE750 group suggested that the ethanol extract of porcupine tail meat might have the potency to activate penile erection since Agmo (1997) said that intromission is not possible without adequate erection and coordinated activity of penile muscles.

Another interesting finding was observed by comparing the trends of the negative control and EE150 group. The negative control is a group where no or lower phenomenon in particular experiment is expected. However, the EE150 group showed lower mounting and intromission frequency compares to negative control. It was also observed that male mice in

this group were relatively passive although the female was constantly approaching them. This result could be an indication of some probabilities. For example, the carrier solution (5% Tween 80) used in the experiment could influence the expected effect from ethanol extract of which the combination of 5% Tween 80 with the low dosage of ethanol extract resulted in a reverse effect. This phenomenon probably was an example of antagonism reaction wherein two or more agents in combination have an overall effect that is less than the sum of their individual effects. This phenomenon might also happen due to failure to produce reaction as the dose was below minimum effective dose. Different carrier solution that commonly used and could be employed for further improvement of the experiment is sodium CMC (Sharma *et al*, 2011).

Table 2. showed the time of ML and IL of male mice in all groups of the experiment. Since a group of EE150 did not show intromission thus only mount latency that can be recorded and it is the slowest time of all groups. Slower ML of ethanol extracts groups compared to control groups might indicate that the extract could not stimulate the motivation to initiate sex. The intromission latency of EE750 group was lower than the negative control group. However, the ethanol extract could not reduce the ML and IL as low as positive control group. Sildenafil citrate was known for its ability to improve penile erection in the erectile dysfunction through increasing levels of cGMP resulted in smooth muscle relaxation (Moreland *et al*, 1999). In this study, sildenafil citrate was used only as a reference for quantitative comparison and not for comparing the mechanisms.

Table 2. Effects of ethanol extracts of porcupine tail meat on mounting and intromission latency of male.

Group	Substance and Dose	Latency (second)	
		Mounting	Intromission
Negative	5 % Tween 80	2092.20 ± 837.86	3259.60± 1532.67
Positive	Sildenafil citrate 5 mg/kg	454.60 ± 305.94	737.20 ± 378.74
EE150	Ethanol Extract 150 mg/ kg	3670.40 ± 2286.78	-
EE750	Ethanol Extract 750 mg/ kg	3150.60 ± 1831.03	2662.60 ± 1345.73

CONCLUSION

Present findings provide preliminary evidence of ethanol extract from porcupine tail meat as an aphrodisiac substance. Sexually inexperienced male mice group treated with ethanol extract of porcupine tail meat at the dose 750 mg/kg BW showed higher mounting and intromission frequency compared to the group of ethanol extract dose 150 mg/kg and after 2 hours of administration showed the highest frequency compare to all groups. However, the ethanol extract could not reduce the mounting and intromission latency as low as sildenafil citrated treated group.

REFERENCES

Agmo, A., (1997). Male rat sexual behaviour. *Brain Research Protocols*, *1*(2), 203-209.

Anita, S. Farida, W. R., Agusta, A. & Nugroho, H. A. (2014, December 22-23). Nutritional components from the tail meat of Sunda porcupine (*Hystrix Javanica* F. Cuvier, 1823) that could be related with aphrodisiac potency. Paper presented at the 4th International Symposium for Sustainable Humanosphere, Bandung.

Asuntha, G., Prasanna, R. Y., Harini, C. V., Vandan, K. R., Arun, R. & Prasad, K. V. S. R. G. (2014). Pharmacological profiling of Argemonemexicana for its aphrodisiac potentials in male Wistar rats. *Asian Pacific Journal of Reproduction*, *3*(2), 110-115.

Hashim R., Roslan, N. A. E. M., Zulkipli, F. H. &Daud, J. M. (2014) Screening of aphrodisiac property in sea slug *Aplysiadactylomela*. *Asian Pacific Journal of Tropical Medicine*, *7*, S150-S154

Krychman, M. L., Gubili, J., Pereira, L., Holstein, L., & Cassileth, B. (2007). Female sexual enhancers and neutraceuticals. *Current Sexual Health Reports*, *4*, 177−182.

Melnyk, J. P. &Marcone, M. F. (2011). Aphrodisiacs from plant and animal sources - a review of current scientific literature. *Food Research International*, *44*, 840-850.

Migdal, W. &Zivkovic.B. (2007).Meat from functional food to diseases of modern civilization.*Biotechnology In Animal Husbandry*, *23*, 19-31.

Moreland, R. B., Goldstein, I., Kim, N. N. & Traish, A. (1999). Sildenafil citrate, a selective phosphodiesterase type 5 inhibitor: research and clinical implications in erectile dysfunction. *Trends in endocrinology & metabolism*, *10*(3), 97-104.

Nurjanah, S., Gumbira-Sa'id, E., Syamsu K. &Riani, S. E. (2009). Effect of sandfish (*Holothuriascabra*) steroid extract on the sexual behaviour and the blood testosterone level of the male mouse (*Mus musculus*). *Majalah Kedokteran Bandung*, *41*, 135-141.

Pande, M & Pathak, A. (2009). Effect of ethanolic extract of *Ocimum gratissimum* (ram tulsi) on sexual behaviour in male mice. *International Journal of PharmTech Research, 1*(3), 468-473.

Prasetya, E. (2012). Daging landak bikin "senjata" loyo jadi perkasa. [Online]. Diambil dari https://www.merdeka.com/peristiwa/daging-landak-bikin-senjata-loyo-jadi-perkasa.html. [2 December 2016].

Ridzwan, B. H., Hanani, M., Norshuhadaa, M. P. S., Hanis, Z. F. & Aileen, T. S. H. (2013). Screening for aphrodisiac property in local oyster of *Crassostreairedalei*. *World Applied Sciences Journal*, *26*(12), 1546-1551.

Sandroni, P. (2001). Aphrodisiacs past and present: a historical review. *Clinical Autonomic Research*, *11*(5), 303−307.

Shamloul, R. (2010). Natural aphrodisiacs. *The Journal of Sexual Medicine*, *7*(1), 39−49.

Sharma, V., Boonen, J., Chauhan, N. S., Thakur, M., Spiegeleer, B. D., Dixit, V. K. (2011). *Spilanthes acmella* ethanolic flower extract: LC–MS alkylamide profiling and its effects on sexual behavior in male rats. *Phytomedicine*, *18*, 1161-1169.

Soxhlet, F. (1879). Die gewichts analytische Bestimmung des Milchfettes. *Dingler's Polytechnisches Journal* (in German), *232*, 461–465

Swaney, W. T., Dubose, B.N., Curley, J.P. & Champagne, F. A. (2012). Sexual experience affects reproductive behavior and preoptic androgen receptor in male mice.

Hormones and Behavior, *61*, 472-478.

Wagner, G., Fugl-Meyer, K. S. & Fugl-Meyer, A. R. (2000). Impact of erectile dysfunction on quality of life patient and partner perspectives. *International Journal of Impotence Research*, *12*, 144-146.