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Time of Maximum Action of Leaf-Methanol Extract of *Pseudopanax Arboreus* (Araliaceae) on the Sexual Activity of Amitriptyline-Induced Sexually Impaired Male Rats

Egbe B Besong*[1], Ateufack Gilbert[2], Koyeu Tatou Emmanuel[3] and Albert Kamanyi[2]

[1]*Department of Zoology and Animal Physiology, Faculty of Science, Cameroon*

[2]*Department of Animal Biology, Animal Physiology and Phytopharmacology Laboratory, , Cameroon*

+Department of Plant Biology, Faculty of Science, Cameroon

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***Corresponding author:** Egbe B Besong, Department of Zoology and Animal Physiology, Faculty of Science, Cameroon

Abstract

The time of most intense action of the leaf-methanol extract of *Pseudopanax arboreus* on the sexual activity of amitriptyline-induced sexually impaired male rats was evaluated using the number of ejaculatory series produced within every 30 minutes of observation. The results showed that *P. arboreus* produced increased number of ejaculatory series between the 60th and 89th minute of observation on the 14th day of treatment. These findings provide further evidence that the leaf-methanol extract of *P. arboreus* increases aphrodisiac potency in sexually impaired male rats.

Keywords: Time; Intense Action; *Pseudopanax arboreus*; Sexually Impaired

Introduction

Leaves of *Pseudopanax arboreus* have been employed traditionally for male potency and virility for almost a century by the people of the Bayang Tribe of the Manyu Division of Cameroon without any scientific investigation. According to their folk medicine, its leaf-maceration is used to improve on male sexual performance or treat male sex-related ailments such as Erectile Dysfunction (ED), ejaculatory dysfunction, disorders of sexual desire and low sperm count by administering a total daily volume of 250 ml of maceration of 20 leaves to an adult male. In previous studies, we evaluated the effects of its leaf-aqueous extract on the sexual behaviour of normal male rats [1] and the effects of the leaf-methanolic extract on the sexual activity of amitriptyline-induced sexually impaired male rats [2]. In the present experiment, we aimed at determining the time of maximum action of the leaf-methanol extract of the plant on the sexual activity of amitriptyline-induced sexually impaired male rats using the number of ejaculatory series produced within every 30 minutes of observation.

Materials and Methods

Plant Material

Fresh leaves of *P. arboreus* were harvested from Mamfe and authenticated with the previous sample deposited at the National Herbarium in Yaounde at the voucher number 2734/SRFK (YA). Meanwhile, the extract was processed and administrative doses determined as described in our previous study [2].

Animals

Animals used were rats of the Wistar Strain of either sex that were bred in the Animal House of the Department of Zoology and Animal Physiology of the Faculty of Science, University of Buea under standard conditions of temperature, humidity and light (12H cycle). Females used as stimuli were brought into estrus following ovariectomy and subsequent sequential injection of 66.67µg of estradiol benzoate and 600µg of progesterone solutions, with the 2 injections separated by 48 ours and the progesterone administered 6 hours prior to observations [1-7]. The research protocol was approved by the University of Buea Institutional Animal Care and Use Committee (UB-IACUC) and an ethical clearance number (UB-IACUC No 003/2018) was given.

Experimental Design

Sexual impairment was induced according to the method described by Neelesh et al. [8] and like in our previous study [2]. Briefly, sexually trained males were subjected to chronic (56 days) administration of an oral dose of 10 mg/kg body weight of

amitriptyline hydrochloride suspension (suspension prepared daily in distilled water). At the end of the 8th week (on day 57) after treatment with amitriptyline, they were randomly selected, their sexual performance assessed and only those that showed low libido, low arousal and delayed ejaculation were recruited into the next phase of the experiment. They were then divided into 4 groups of 8 rats each and treated as follow: groups 1 and 2 received 10ml/kg distilled water and 6mg/kg of Viagra to serve as the neutral and positive controls respectively; while groups 3 and 4 were administered 46.5 and 93mg/kg of the leaf-methanol extract of *P. arboreus*. Males were housed singly in standard propylene cages and 30 minutes after administration of either substance, an estrous female was introduced into the cage and the sexual activity of the male evaluated. Treatment lasted for 3 calendar weeks (21 days) with sexual performance evaluated at the end of each week. The number of ejaculatory series produced within each 30 minutes of observation were noted. Each observation session lasted for 120 minutes but was considered terminated once the Mount Latency (ML) or Post Ejaculatory Interval (PEI) was 20 minutes.

Statistical Analyses

Values were expressed as Mean ± SEM. Mean values were calculated for each animal and quantitative comparisons between groups established from those means. Analysis of Variance (ANOVA) repeated measures followed by Duncan test were done using SPSS for windows version 20.0. Significant levels were tested at $p<0.05$.

Results and Discussion

The time-effects of the leaf-methanol extract on the sexual performance of sexually impaired male rats are shown in Table 1. Generally speaking, although withdrawal of the drug resulted in improvement on the sexual activity, treatment with the leaf-methanol extract induced a significant ($p<0.05$) increase in sexual performance, with a maximum effect noted on day 14 of treatment between the 60th and the 89th minute of observation. As shown in the Table, while sexual performance of extract-treated animals improved in a dose-dependent manner from day 1 through day 21 of treatment, the reverse was noticed with the Viagra-treated animals, whereas no significant change was noticed in distilled water-treated rats. The decrease in effect with duration of treatment noticed in Viagra-treated rats could be due to pharmacodynamic tolerance, which occurs when the same concentration at the receptor site results in reduced effect with repeated exposure [9,10]. On the contrary, the leaf-methanol extract of *P. arboreus* dosed at either 46.5 or 93mg/kg daily can be said to take 2 weeks or 14 days to reach full effect. This can be explained by the delay in the turnover of mediators of its actions that each probably has a half-life of several days. The physiological actions of the plant extract are a consequence of cumulative drug action [11,12].

Table 1: Number of ejaculatory series produced by amitriptyline-induced sexually impaired male rats treated with the leaf-methanol extract of *Pseudopanax arboreus*.

Week	Period of Observation	Number of Ejaculatory Series/Treatment			
		DW (Neutral Control)	Viagra (Positive Control)	ME 46.5mg/Kg	ME 93mg/Kg
1 (1-7days)	0-29 minutes	1.38±0.13	5.69±0.47^{A}	3.82±0.17	3.96±0.44
	30-59 minutes	1.22±0.14	5.85±0.44^{A}	3.87±0.16	4.12±0.43
	60-89 minutes	1.26±0.13	5.93±0.51^{A}	3.62±0.35	4.09±0.46
	90-119 minutes	0.88±0.09	5.64±0.58^{A}	3.96±0.55	3.85±0.34
2 (8-14 days)	0-29 minutes	1.31±0.17	4.67±0.44	2.40±0.25	2.89±0.23
	30-59 minutes	1.23±0.16	4.81±0.42	3.55±0.29	4.08±0.41
	60-89 minutes	0.78±0.08	3.24±0.41	5.33±0.33^{A}	5.61±0.2^{A}
	90-119 minutes	1.29±0.13	3.27±0.37	5.17±0.32	5.43±0.31
3 (15-21 days)	0-29 minutes	1.23±0.11	4.77±0.63	5.13±0.35	5.22±0.34
	30-59 minutes	1.11±0.13	4.81±0.66	5.06±0.39	5.29±0.37
	60-89 minutes	1.14±0.08	4.66±0.57	5.18±0.37	5.24±0.41
	90-119 minutes	1.19±0.08	4.70±0.64	5.20±0.34	5.26±0.42

Note: Mean ± SEM; DW: Distilled Water; ME: Methanol Extract; A: Significant when compared to the neutral control (distilled water); $p<0.05$.

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