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Phase-dependent effects of purwoceng (*Pimpinella alpina* KDS) on estrous cycle modulation in virgin female rats

Pudji Achmadi, Damiana Rita Ekastuti, Ronald Tarigan, Isdoni Bustaman, Koekoeh Santoso, Hera Maheshwari, Aryani Sismin Satyaningtijas*

Division of Physiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia

ABSTRACT: Purwoceng (*Pimpinella alpina* KDS) is a traditional medicinal herb that has been widely studied for its androgenic properties in male reproductive research. However, their effects on female reproductive performance remain poorly understood. This study aimed to investigate the influence of purwoceng on the duration of each estrous phase and the overall estrous cycle in female rats. Forty-eight virgin female Sprague-Dawley rats were allocated into four groups according to their estrous phase at the start of treatment: proestrus, oestrus, metestrus, and diestrus. Each group consisted of six control and six Purwoceng-treated animals. The extract was administered intragastrically for ten consecutive days, while vaginal smear evaluations continued until day 15 to monitor estrous cyclicity. The results demonstrated that purwoceng administration during the two estrous cycles generally prolonged the proestrus and oestrus phases, with a statistically significant extension of the oestrus phase observed when treatment was provided during oestrus ($P < 0.05$). Conversely, the durations of the metestrus and diestrus phases were reduced, except when Purwoceng was administered during metestrus, which resulted in an increased metestrus duration. In conclusion, purwoceng exhibited the greatest modulatory effect when administered during the oestrus phase, suggesting a phase-dependent responsiveness of the female reproductive cycle to this herbal extract.

Keywords:
oestrous cycle, purwoceng, reproduction, female, virgin rat

■ INTRODUCTION

The reproductive-enhancing properties of purwoceng have been widely explored in males. Previous studies have reported that the administration of purwoceng extract to male Sprague–Dawley rats increased luteinizing hormone (LH) and testosterone levels, improved sperm motility, and enhanced spermatogenesis (Juniarto 2010). Phytochemical screening has further identified the presence of steroid, triterpenoid, alkaloid, and flavonoid compounds in this plant, supporting its potential bioactivity in reproductive function (Timotius *et al.* 2023).

While the androgenic effects of purwoceng in males have been documented, its effects on female reproduction remain unexplored. Testosterone may be converted to oestrogen via aromatase in granulosa cells, suggesting purwoceng's potential female reproductive effects. Limited evidence exists on the influence of purwoceng root extract on estrous cycles and ovarian activity. This study evaluated the reproductive effects of purwoceng in early pubertal female rats by investigating its influence on estrous cycle duration and phase length.

■ MATERIALS AND METHODS

Female rats (150–200 g) were assigned to four groups according to the estrous cycle stage. Classification was based on predominant cell types in vaginal smears: proestrus (P) when >75% were nucleated epithelial cells; oestrus (E) when >75% were cornified epithelial cells; metestrus (M) when >75% were leukocytes; and diestrus (D) when leukocytes and nucleated epithelial cells were equal. Each phase included 12 rats, divided into control and Purwoceng treatment groups of six animals each.

The treatment group received purwoceng extract at a dose of 83.25 mg/kg body weight for ten consecutive days. Vaginal smears were collected daily for 15 days to monitor changes in the estrous cycle length and duration of each phase. All animals were maintained alive throughout the experiment, and no euthanasia procedures were conducted. The rats were housed in the Animal Facility of the Unit Pengelola Hewan Laboratorium, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia, under standard husbandry and welfare conditions.

■ RESULTS AND DISCUSSION

The length of the estrous cycle and the duration of each phase in rats force-fed purwoceng are presented in Table 1. Rats receiving purwoceng during the proestrus and oestrus periods tended to exhibit longer proestrus and oestrus phases than the

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controls. Notably, the oestrus period was significantly prolonged in the treated rats ($P < 0.05$). A longer oestrus duration increases the window of sexual receptivity, thereby enhancing the likelihood of mating and, consequently, the probability of successful fertilisation.

Force-fed rats during proestrus and oestrus showed shorter metestrus and diestrus periods than controls. Rats treated during the metestrus and diestrus phases showed lengthened proestrus and oestrus phases, except for rats treated in diestrus, where proestrus duration matched controls. Treatment during metestrus and diestrus led to shorter durations of later cycle phases, except for metestrus, which was prolonged when purwoceng was administered. The overall estrous cycle length remained similar between the treated and control groups.

Phytochemical analysis by the Medicinal and Aromatic Plants Research Institute (Rusmin & Darwati 2018) showed that purwoceng roots contain alkaloids (+++), tannins (+), flavonoids (+++), triterpenoids (+), steroids (+), and glycosides (+), while saponins and phenolics were absent. The strong presence of alkaloids and flavonoids with weakly positive steroids was notable. These compounds act as phytoestrogens by mimicking endogenous oestrogenic activity, binding to oestrogen receptors with lower affinity and reduced potency. The major phytoestrogen classes in plants include isoflavones, flavones, lignans, coumestans, triterpenes, and acyclic compounds (Arjadi *et al.* 2024).

The prolongation of proestrus and oestrus in treated rats may be due to elevated testosterone levels after Purwoceng administration. Testosterone can be aromatised into oestrogen via granulosa cell aromatase, increasing oestrogen concentration. The active constituents of purwoceng likely bind to LH receptors on theca cells and/or androgen receptors on granulosa cells, thereby modulating the steroidogenic pathways. Oestrogen receptors ERα and ERβ are located in the target cells of the uterus, anterior pituitary, mammary gland, and reproductive tissues. ERα functions mainly in uterine tissues, whereas ERβ is prominent in the ovary (Chen *et al.* 2022). Owing to the hydrophobic nature of oestrogen, it diffuses into cells and interacts with cytoplasmic receptor proteins.

Phytoestrogens share a structural similarity with 17β-estradiol, enabling them to bind to the oestrogen receptor alpha (Canivenc-Lavier & Bennetau-Pelissero 2023). This receptor-binding capacity supports the hypothesis that purwoceng influences the follicular phase, comprising proestrus and oestrus, through oestrogenic or oestrogen-modulating activity.

Conversely, metestrus and diestrus constitute the luteal phase, during which progesterone secretion from the corpus luteum predominates. Because progesterone-driven processes are less dependent on oestrogenic activity, purwoceng appears to exert minimal influence during this phase, which is consistent with the relatively unchanged patterns observed in treated rats.

Table 1. The average value of the length of the oestrous cycle and its period in virgin rats

Period of treatment	Observation (hours)				Length of cycle
	P	E	M	D	
P					
Control	12±0.0	12±3.1	24±0.0[b]	68±3.5	116±1.6[b]
Treatment	12±0.0	16±6.9	20±3.5[a]	58±6.9	106±4.3[a]
E					
Control	12±0.0	16±3.5	22±6.9	70±6.9[b]	120±4.3[b]
Treatment	12±0.0	20±3.5	18±0.0	56±3.5[a]	106±1.9[a]
M					
Control	12±0.0	12±0.0	24±0.0	70±3.5[b]	118±0.9[b]
Treatment	12±0.0	14±3.5	26±3.5	56±6.9[a]	108±3.5[a]
D					
Control	12±0.0	12±0.0	26±3.5	66±6.0	110±0.9[b]
Treatment	12±0.0	14±3.5	22±3.5	60±6.0	108±1.7[a]

Note: [a,b]different letters in the same column and row indicate there is significant difference ($P<0.05$); P: Proestrous, E: Estrous, M: Metestrous, D: Diestrous, Control: no treatment, Treatment: treated with purwoceng

■ CONCLUSION

Administration of purwoceng across the two estrous cycles resulted in an overall prolongation of the proestrus and oestrus phases, with a statistically significant extension of the oestrus phase observed when the treatment was administered during oestrus ($P < 0.05$). In contrast, purwoceng generally shortened the metestrus and diestrus phases, except when administered during metestrus, in which case it produced a significant increase in the duration of the metestrus phase.

■ AUTHOR INFORMATION

Corresponding Author

*ASS: aryanisa@apps.ipb.ac.id

Division of Physiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Jln Agathis, Kampus IPB Dramaga, Bogor, 16680, West Java, INDONESIA.

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