

Effects of crude root extract of *Polygonum hydropiper* on estrous cycle and induction of reversible sterility in female albino rat

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Summary

Polygonum hydropiper is a wild plant found in Assam, a North-Eastern province of India. Tradition prevails among the folk women of Assam to use the root of this herb for fertility control. In the present investigation crude methanolic extract of root of this plant was tested for anti-fertility property in female albino rat. Adult cycling female rats were administered through oral route the crude root extract at a dose of 1 g / kg body weight / day for 12 days. Subsequently, the rats were allowed for recovery until day 63. The estrous cycle was monitored routinely through analysis of the vaginal smear. On termination of treatment the rats were allowed to mate with males and to complete the full term of gestation. The number of newborn pups was considered as the number of implantations. The estrous cycle of the extract treated rats became irregular, resulting in failure of gestation. The estrous cycle was restored following the recovery period. However, the number of newborn pups was significantly lesser than in the controls. The results reveal that the root of *Polygonum hydropiper* contains steroidal / estrogenic compound(s) which can affect the female reproduction in rat.

Key Words: *Polygonum hydropiper*, endocrine disruption, reversible infertility

Introduction

Polygonum hydropiper is a herb widely distributed in the damp and swampy areas of North-Eastern part of Indian subcontinent. Assam is one of the North-Eastern States of India, inhabited by different ethnic groups enriched with indigenous knowledge on traditional medicine. Tradition of using the root of *Polygonum hydropiper* for fertility control by the folk women prevails among some of these ethnic groups. In order to find if there is scientific basis in this use, the crude root extract of the plant was tested for effect on implantation and estrous cycle in female albino rat. Earlier, this plant has been reported to be in use in Europe to correct menstrual irregularities (Kirtikar and Basu, 1935) as well as for fertility regulation (Weed, 1986). Earlier findings from this laboratory showed that the crude root extract of this plant mimics estrogenic effect (Hazarika and Sarma, 2006a) and, through this effect, can modulate endometrial cellular organization in female rat (Hazarika and Sarma, 2006b). It has been hypothesized that root of *P. hydropiper* contains phytosteroid(s), which would affect female reproduction of albino rat and the effect would be through interference with the implantation process with or without disturbing the endocrine system. The finding

showed that the crude root extract disrupts the estrous cycle of rat and induces reversible infertility.

Materials and Methods

Preparation of root extract

The roots of locally available *Polygonum hydropiper* was collected, washed and shade-dried. The dry root was chopped into small pieces and powdered in a mixer grinder to about 60 mesh size. The powder was soaked in methanol for 72 hr at room temperature ($25 \pm 2^\circ\text{C}$) and, subsequently, filtered. The filtrate was concentrated under vacuum at $27 \pm 2^\circ\text{C}$ and stored at -20°C until use.

Experimental animal

Adult cycling female albino rats (150 ± 10 g body weight) were used. The animals were kept under uniform husbandry conditions of natural light and temperature and fed with the pellet feed and water *ad libitum*. The estrous cycle of the rats was assessed by observation of vaginal smear. The rats showing only the normal estrus cycle (95–105 hr) were selected for the study.

Administration of crude root extract (CRE)

The extract was suspended in normal saline and administered to the rats through oral route at a dose of 1 g / kg body weight / day between 08.00 - 09.00 hr for a duration covering three consecutive cycles (12 days) starting from the onset of proestrus, according to Hazarika and Sarma (2006a, b). The control rats were treated with normal saline.

Monitoring of estrus cycle

The estrous cycle of both control and treated rats was monitored through observation of cell types in the vaginal smear according to Montes and Luque (1988). Vaginal fluid was collected with the help of smooth dropper filled with normal saline and placed on a slide to prepare a smear and allowed to air dry. After fixation in methanol for 4 to 5 min the slides were stained in Geimsa's for 5 to 6 min. Finally, the slides were washed in tap water, dehydrated in 95% and 100% alcohol, cleared in xylene and mounted in DPX mountant.

Experimental design

To understand the effect of crude root extract (CRE) on female reproduction, presence of characteristic cell types in the vaginal smear was considered as the index. Adult cycling female rats were administered through oral route the CRE for 12 days. The rats were allowed to mate with the male of proven fertility from day 13 onwards of post-treatment. Mating was confirmed from the presence of spermatozoa in the vaginal smear. The estrous cycles were monitored from day 1 of treatment through the period when the female allowed the male to mate. The latter was considered as the day of mating and the first day of gestation. The females were allowed to complete the full term. The number of pups given birth to was considered as the number of implantations.

Results

Effect on estrous cycle

The vaginal smears of control rats during the different stages of the estrous cycle were representative of the respective stages (Fig. 1). Administration of CRE of *Polygonum hydropiper* to female albino rats induced alterations in the vaginal smear (Fig. 2). There was deformation of the cell structure from day 13 onwards and there were only fewer cells than in the control rats. Acute alteration in the vaginal smear was recorded from day 35 onwards, when the least number of cells appeared. Moreover, the cell types in the vaginal smear were not characteristic of the respective stages. During the proestrus

the karyopyknotic cells appeared deformed. A few intensely staining cornified cells were present but not similar to those in the control rats at the corresponding stage. During the luteal phase (metestrus and diestrus) there were very few cells, which did not possess the characteristic pattern of polymorphs (Fig. 2C, D).

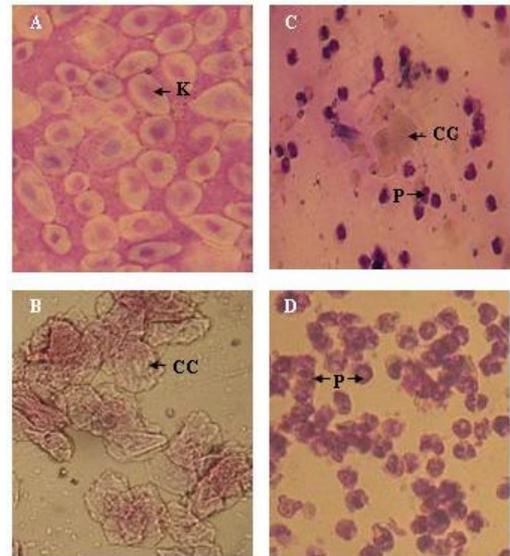


Fig. 1. Photomicrographs of cells types in the vaginal smears of cycling female albino rats (control). Normal estrous cycle is characterized by presence of karyopyknotic cells (K) during proestrus. A, proestrus; B, estrus; C, metestrus; D, diestrus. CC, cornified cells; P, polymorphonuclear leucocytes (Giemsa's; x40).

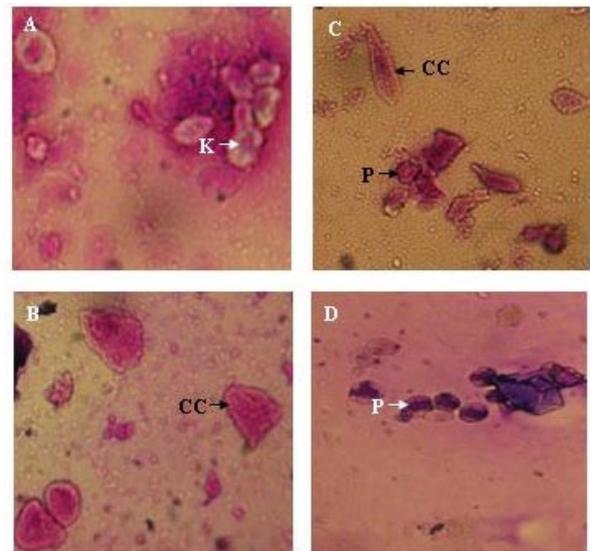


Fig. 2. Photomicrographs of altered cell types in vaginal smear during abnormal cycle following CRE treatment (35 days). The cell types characteristic of each stage are not there, and the abundance of cells is much fewer. A, proestrus; B, estrus; C, metestrus; D, diestrus. K, karyopyknotic cell; CC, cornified cell; P, polymorphs (Giemsa's; x40).

The CRE treated rats recovered gradually, as revealed in the vaginal smears, from day 44 onwards, and complete recovery was recorded on day 61 (Fig. 3). The treated rats became receptive to the males following this period of recovery.

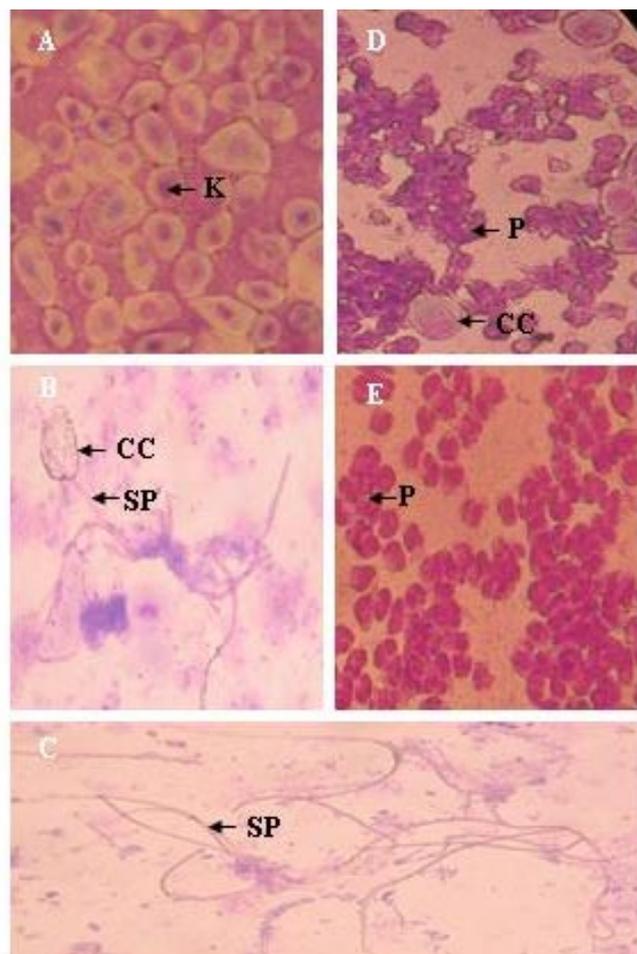


Fig. 3. Photomicrographs of cell types in the vaginal smears of CRE-treated female rats during posttreatment period following recovery of estrous cycle (61days). The cell types characteristic of the respective stages are found, indicating complete restoration of the reproductive cycle. Presence of spermatozoa (SP) confirms mating during this period. A, proestrus; B, estrus; C, metestrus; D, diestrus. K, karyopyknotic cells; C, cornified cells; P, polymorphonuclear leucocytes; SP, spermatozoa.

Reversibility and litter size

All the untreated rats became pregnant when allowed to mate with male rats of proven fertility. All the CRE treated rats become pregnant after the recovery. However, the litter size was significantly lesser in the treated rats that had recovered than control rats (Fig. 4).

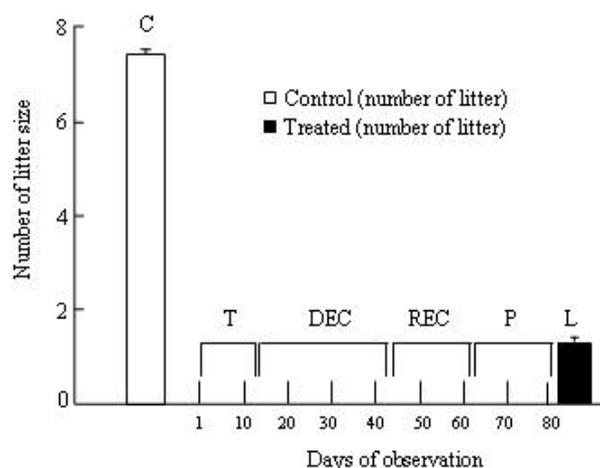


Fig. 4. Graphical representation of effect of CRE on implantation, induction of infertility, reversibility, loss of cyclicity and recovery of normal estrous cycle. C, Control females. Treatment started from day 1 of proestrus to 12 days (T). Disrupted estrous cycle (DEC) was observed from day 13 to day 43. Estrous cycle was recovered (REC) from day 44 onward to day 60. The treated females became pregnant (P) from day 61 to day 80. Litter size (L) during posttreatment period was significantly lower than in the control. Values are mean \pm S.E. (Sample size = 12)

Discussion

The presence of particular cell types in the vaginal smear indicates the follicular and luteal phases of the reproductive cycle. In female reproduction, uterine endometrium is one of the target organs of gonadal steroids, which in turn is dependent on the hypothalamo-hypophyseal-gonadal axis (Turner and Bagnara, 1975). The gonadal steroids, estrogen and progesterone, can modulate the uterine tissues, induce endometrial maturation and render endometrium receptive to blastocysts (Dey et al., 2004). The role of estrogen in endometrial cellular proliferation is well established (Snijders et al., 1992; Ashby et al., 1999). Disruption of estrous cycle may cause the disparity of endometrial function and *vice-versa*. The structural and functional changes of the endometrium may lead to failure of implantation. In the present study, it was observed that administration of CRE of *Polygonum hydropiper* not only affected the estrus cycle but also the implantation process. The precise mechanism of the action of CRE on suppression of implantation is not known. It is speculated that CRE induces changes in the biochemical profile of the uterine tissue, preparing the latter unfavorable for implantation. Alternatively, CRE may affect the ovarian follicular development and hinder the process of ovulation resulting in failure of pregnancy (Hazarika and Sarma, 2006b).

The results of the study suggest that CRE can alter the ovarian endocrine function resulting in a thorough change in the characteristic cell types in the vaginal smear during the posttreatment period (day 13-43). The loss of cyclicity indicated disruption of ovarian estrogen and progesterone, which are the essential for normal uterine endometrial maturation and receptivity to the embryo. The dose of CRE practiced was capable of inducing infertility from day 13 onwards. With the resumption of the estrous cycle of the CRE-treated rats from day 43 onwards, the animals recovered from the infertility. The treated females allowed mating and become pregnant. However, the litter size was significantly less compared to the untreated rats. The precise mechanism of CRE of *Polygonum hydropiper*-induced irregularity of the reproductive cycle is not clearly understood. It is speculated that although estrous cycle is restored 22 days after cessation of CRE treatment and the females allowed the males to mate, the cellular and/or biochemical milieu of the endometrium was not conducive for the embryo to implant. Also, the ovarian follicular development and ovulation appear to have been hampered as revealed in the smaller litter size and, therefore, implantation. This corroborates our earlier study in which we found many of the Graafian follicles in the treated ovary showed pyknosis of the nuclei of granulosa and theca cells, as well as disparity in granulosa cell organization. In the uterine tissues CRE induced hyperplasia in places of luminal epithelium and degeneration of endometrial glands (Hazarika and Sarma, 2006b). This functional aberration of the ovarian and endometrial tissues may be mediated through disruption of hypothalamo-hypophyseal-gonadal axis of hormone secretion. These putative structural and/or functional alterations of the reproductive organs by CRE would have led to the lesser number of implantations after the recovery. The precise cause and mechanism underlying the decreased litter size remains to be investigated.

It may also be speculated that CRE would induce changes in the secretion of the endometrial glands too. During the pre-implantation period and/or early pregnancy in the rat, endometrial glands synthesize and secrete several proteins required for establishment of uterine receptivity and embryo implantation (Stewart and Cullinan, 1997; Carson et al., 2000; Allison et al., 2001). Due to administration of CRE of *Polygonum hydropiper* to rat, synthesis of some endometrial proteins is altered (Hazarika and Sarma, 2006a), which might affect the normal cyclicity as well as restoration of normal implantation process. The antifertility effect of phytochemicals on female reproduction through unspecified mechanisms has been shown by many

investigators (Devarshi et al., 1991; Ratnasooriya et al., 1994; Upadhyay et al., 1994; Goonasekara et al., 1995). We speculate that the phytosteroid(s) alone or in combinations with other phytochemicals would have induced infertility in the female (Hazarika and Sarma, 2006b). However, the precise mechanism of action of CER in bringing about this effect remains to be elucidated.

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