



Effect of Ethanolic Extract of *Aristolochia indica* on the Oestrous Cycle of Adult Rats

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Received: 10-05-2019; Revised: 22-06-2019; Accepted: 02-07-2019.

ABSTRACT

Rapid population growth is becoming a problem which causes severe pressure on economic, social and cultural resources. Control of fertility using traditional antifertility plants has been practiced for many years in Africa including Ethiopia. In the present study the anti-implantation and abortifacient property of crude extract of the leaves of the plant *in vivo* and the spasmogenic effect of its fractions in isolated mouse uterus (MU) *in vitro* were tested. The effect of ethanolic extract of *Aristolochia indica* has been studied on the duration of oestrous cycle in adult rats. The extract of 150 mg/kg dose induced cornified phase at the initial days of treatment which persisted till the last day of treatment. Induction and persistence of cornification in the oestrous cycle, due to the treatment of ethanolic extract has been accounted for its estrogenic nature.

Keywords: *Aristolochia Indica*, *in vivo*, anti-fertility, oestrous cycle, female rats.

INTRODUCTION

According to World Health Organization (WHO, 2002), traditional medicine is defined as diverse health practices, approaches, knowledge and beliefs incorporating plant animal and /or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being as well as to treat, diagnose or prevent illness.

Number of reports postulate the potent antifertility effect of *Aristolochia indica* in laboratory animals. Further- more, its possible way of anti-implantation action has been described by virtue of its antifermultiple hormonal properties. Our recent findings report that the butanolic fraction of the tubers of *Aristolochia Indica* is most effective to prevent implantation in rats where 150 mg/kg body weight dose per day has been considered as the minimum effective dose in 7 days schedule (1-7 post *coitum*). It is well established that antifertility agents acting on the ovarian-uterine axis certainly provoke changes in the pattern of reproductive cycles. Although the ethanolic extract of *A. Indica* is known to possess significant estrogenic, antiestrogenic and progestational activities at different dose levels but its effect on the periodicity of oestrous cycle is not yet known.¹ The work to be reported here has therefore, been initiated in hope to evaluate the hormonal dependent effect of ethanolic extract of *A. Indica* on the duration of various phases of the cycle in adult intact rats.

MATERIALS AND METHODS

Fresh tubers of *Aristolochia Indica* were collected from Shivpuri (M.P.). They were chopped, dried in shade, powdered and extracted with ethanol as described earlier. Colony bred Swiss mature female rats (150 & 10 g) were used for the present study. These animals were kept under

uniform husbandry conditions of light and temperature and were fed with 'Hindustan Lever' Gold Mohur rat pelleted diet and water *ad libitum*. The vaginal smear of rat was examined daily at 10.00 AM for 18-20 days to select animals of regular cycles. When the rats showed proestrus phase, the normal cyclic animals were randomized into different groups (Table 1).

Ethanolic extract of *Aristolochia indica* was macerated with gum acacia suspended in distilled water and a dose of 150 mg/kg body weight was prepared. It was administered orally with the help of intra gastric catheter to 4 different groups of animals for 6, 12, 18 and 24 days separately. Parallel controls were run with each group which received gum acacia suspension as vehicle. Throughout the experimentation, the vaginal smear of each rat was checked daily at a regular interval of 24 hours. At the end of the experiment, the record of the different stages of the oestrous cycle of each rat was analysed.²

Table 1 shows the effect of ethanolic extract of *A. Indica* on the duration of various stages of the estrous cycle in adult rats. It reveals that in control animals the estrous cycle is regular and normal. Various stages of the cycle viz. proestrus, estrus, metaestrus and diestrus revolve in cyclic clockwise direction (Fig.1). The percent phase duration of each stage remains static irrespective of their cycles of shorter days (6 days) and longer days (24 days).³ The administration of ethanolic extract at 150 mg/kg body weight/day to intact cyclic adult rats significantly lengthened the duration of cornified phase of the estrous cycle. The extension in the estrus phase is applicable to all the durations of treatment; however, there is a gradual increase from 6 to 24 days of the treatment.⁴ The prolongation of the estrus phase in the treated rats caused suppression in the duration of other stages like metaestrus



and diestrus. Proestrus phase was completely abolished (Fig. 1).

Table 1: Effect of ethanolic extract of *Aristolochia indica* on the duration of various stages of estrous cycle in rats (n=6 in each group)

Duration of stages of the oestrous cycle in days (Mean+ SEM)				
Duration of treatment (Days)*	Proestrus	Estrus	Metaestrus	Diestrus
6	1.00±0.00 (0.00±0.00)	2.33±0.25 (5.80±0.22)	1.00±0.00 (0.6±50.27)	3.66±0.25 (1.60±0.28)
12	1.83 ±50.37 (0.00±0.00)	4.16±0.37 (11.40±10.44)	1.66±0.40 (0.6±0.27)	6.33±0.51 (2.00±0.36)
18	2.66±0.46 (0.00±0.00)	5.66±0.51 (17.20±0.22)	2.50±0.61 (0.8 ±0.22)	9.16± 0.58 (2.00±0.00)
24	3.00±0.54 (0.00±0.00)	7.50±0.61 (23.40±0.27)	3.50±0.88 (0.8±0.22)	12.00±0.89 (1.80±0.23)

Duration of stages of the oestrous cycle in days (Mean+ SEM); * The rats were sacrificed after 48 hrs of last treatment and therefore, the analytical data also include 2 additional days.; Figures in parentheses indicate the values in the control animals.

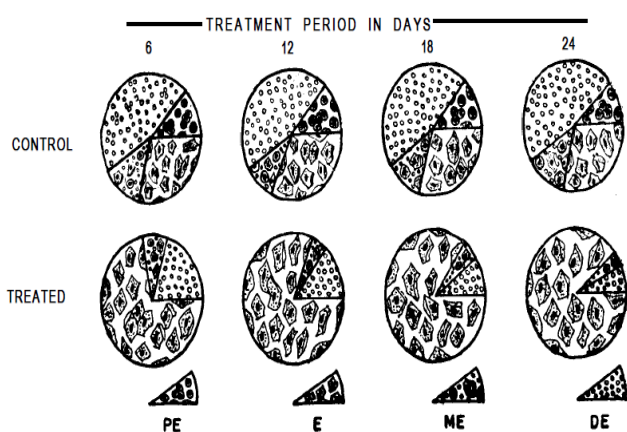


Figure 1: Effect of Ethanolic extract of *A. Indica* on the periodicity of oestrous cycle in rat.

(PE: Proestrus; E: Estrus; ME: Metaestrus; DE: Diestrus)

The changes in the vagina of the normal animals are believed to be due to the fluctuations and inter conversions of female sex hormones, estrogen and progesterone, mainly synthesized in the ovary. The level of these hormones, however, is controlled by the pituitary gonadotrophins and hypothalamic releasing hormones.⁵ A feed- back mechanism also operates whereby the pituitary releases gonadotropins which are in turn controlled by these estrogens and progesterone. The cornification in the vagina is mainly due to the level of stimulation of estrogen

which acts directly on the vaginal epithelium. It is also known that only estrogen consistently stimulates the proliferation of the vaginal epithelium in adult spayed animals.⁶

In the present investigation the ethanolic extract of *A. Indica* at a dose of 150 mg/kg body weight induced the cornified stage which persisted till the last day of treatment.⁷ These findings on the estrous cycle clearly corroborate the potent estrogenic nature of the extract which has already been assessed in immature ovariectomized rats.⁸ Our earlier observations also reveal the antiestrogenic and progestational activities of the ethanolic extract of *A. Indica* at higher dose of 600 mg/kg/day. Progesterone can interrupt the estrus stage of the vaginal cycle but the dose and the days of treatment are the main factors, as it has been observed that single dose of progesterone given to rats at the same time as estrone,⁹ prolongs the effect of estrogen rather to interrupt it. In the present findings we could not observe any interruption in the estrus phase during the entire period of treatment at 150 mg/kg body weight when given for the longer period of 24 days. This further confirms the dose dependent nature of the extract.¹⁰ Further studies are in progress to quantifying the estrogen effect of ethanolic extract of *A. Indica* in rats.

Acknowledgement: Author are thankful to the SSSUTMS, Sehore, and to Dr. Deepak Kumar Mittal for providing guidance.

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Source of Support: Nil, Conflict of Interest: None.

