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***Research Article***

# *POLYGONUM HYDROPIPER* CRUDE ROOT EXTRACT EXERT ESTROGENIC EFFECT ON UTERINE DNA CONTENT IN FEMALE ALBINO RAT

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## ABSTRACT
The root of *Polygonum hydropiper* has anti reproductive property on female albino rat mediating its effect through estrogenic compound(s). The biochemical study showed that the estrogenic effect can change the uterine DNA profile. The results showed that CRE significantly increased the DNA in ovary intact female uterine tissue than that of control females. Similar to the ovary intact in OVX female, administration of CRE increased the level of DNA than that of the control. Significantly the data of CRE treated OVX females is similar to the values of OVX female uterine tissues treated with estradiol – 17 β ($E_2$). The crude root extract of *Polygonum hydropiper* exert its estrogenic effect on the translational level of gene in rat uterine tissue but not transcription.

***Key Words:*** *Crude Root Extract (CRE), Estradiol - 17β ($E_2$), Polygonum hydropiper, Ovariectomized (OVX)*

## INTRODUCTION
Assam being a province of Sub – Himalayan region of North Eastern India is endowed with large varieties of medicinal plants (Garg *et. al.,* 1978). Many of these plants/herbal preparations have been reported to use to control fertility by the indigenous people of this region and tested its effect on female reproduction (Sarma & Mahanta, 2000). While many of such traditional drug prototypes are used in composite forms, some are used individually for reproduction regulation. Most of this traditional drug prototype is crude and used by folk women to control pregnancy. The dry powder of *Polygonum hydropiper* is one of such traditional drugs used by folk women of Assam to control fertility. It is believed amongst the folk women that continuous use of this drug for longer period (12 months) may cause permanent sterility. This information tempted the present investigators to undertake an experimental validation of the root of *Polygonum hydropiper* on female reproduction. Earlier works from this laboratory confirm the estrogenic effect of crude root extract of *Polygonum hydropiper* on protein expression in uterine tissues of female albino rat (Hazarika & Sarma, 2006). In addition, methanolic crude root extract of *Polygonum hydropiper* induces follicular recruitment in the ovary and endometrial hyperplasia similar to the effect of ovarian estrogen (Hazarika & Sarma, 2006). In the present investigation effort has been made to lonk the estrogenic effect of crude root extract of *Polygonum hydropiper* on total DNA content in rat uterine tissue. However, the result showed that crude root extract of *Polygonum hydropiper* exerts estrogenic effect on total DNA content of rat uterus. Therefore, it has been speculated that the roots of *Polygonum hydropiper* contain certain estrogen agonist which mobilized the biochemical component of the uterine tissue partially estrogenic. For in vivo testing the crude methanolic extract of the dry root powder was tested on ovary intact and OVX female albino rat, the data was compared with that of $E_2$ treated OVX female.

## MATERIALS AND METHODS
### *Preparation of root extract*
The roots of locally available *Polygonum hydropiper Linn.* was collected, washed and shade dried. Dry roots were chopped into small pieces and powdered in a mixer grinder. The powdered root was soaked in

methanol for 72 hours at room temperature (25±2$^0$C) and subsequently filtered off. The filtrate was concentrated under vacuum at 27±2$^0$C and stored at -20$^0$C until use.

***Experimental animals***

Adult cyclic female rats (150±10gm body weight) were used for present investigation. Animals were kept under uniform husbandry conditions and natural light and temperature. Rats were fed with routine diet (Bengal gram, corn) and water ad libitum. The estrous cycle of the adult females were studied by observation of cell types in the vaginal smears prior to the experiment. The females showing only the normal estrous cycle (95 – 105hrs) were selected for the in vivo study.

***Experimental design***

The estrogenic property of CRE of *Polygonum hydropiper* was studied in ovary intact control and ovariectomized (OVX) female albino rat. The effect of the CRE on ovary intact females provides information of the effect of CRE on uterine biochemical component in presence of natural source of estrogen. The OVX model was used to study the effect of CRE and estradiol – 17 β ($E_2$) separately on the biochemical component in absence of natural source of estrogen. Estradiol – 17 β was used as the reference drug of CRE in OVX model.

***Ovariectomy of the adult female***

To study the estrogenic property of the root extract of *Polygonum hydropiper*, adult cyclic females were ovariectomised (Hogan *et.al*. 1986). Briefly, intact cyclic rats were subjected to mild Diethyl- Ether anesthesia and ovariectomy was made by two dorso-lateral incision approximately 1 cm long above the ovaries. With the use of sharp dissecting scissors, the skin was cut almost together with the dorsal muscles and the peritoneal cavity was thus accessed. The muscle incision required no suturing. Skin wounds were closed bilaterally with one single catgut suture. Females were allowed to recover for a minimum period of three weeks before starting the experiment.

***Administration of crude root extract (CRE) and estradiol - 17β (E2)***

The CRE of *Polygonum hydropiper* was administered to the adult female rats through the oral route. The CRE was suspended in distilled water and administered to the female rats in a dose of 1000mg/kg body weight /day. Extract was fed to both the ovary-intact and OVX female rats. Administration of the root extract was made for three consecutive cycle (12 days) starting from the onset of proestrus of ovary-intact females and simultaneously to the OVX females for the similar period. Extract was administered during the morning hours in between 8.00 - 9.00A.M. to all groups. The control ovary-intact and OVX females were treated with distilled water in the same manner and for the similar period. Females were sacrificed on the morning of day 13 by cervical dislocation. The Uterine horns were collected and stored at -70$^0$ C until use.

To compare the estrogenic property of the root extract on uterine tissues, a group of OVX female was injected with estradiol -17β (E2). E2 (Lancaster cat No.LO3801, F.W. 272.39) was dissolved in sesam oil in a final concentration of 1.00µg/ml. The OVX females were injected subcutaneously (sc) with 0.1 µg of E2 at an interval of 24 hours for three consecutive days. Another group of OVX female was injected with sesum oil in a similar dose for the similar period and treated as control for E2. The OVX E2 treated and sesum oil treated females were sacrificed following three hours of the last injection. The uterine horns were collected immediately and stored at -70$^0$ C until use.

***Estimation of DNA***

The DNA was extracted following the precipitation and separation of protein by ice-cold TCA. 2ml of 5% tissue homogenate was mixed with 2.5 ml of cold TCA solution in ice bath for 10 minutes and then centrifuged at 4000 rpm for 5 minutes. Supernatant was discarded and the precipitate was washed in 6ml of 95% alcohol followed by another wash with a mixture of 6 ml of dehydrated alcohol and diethyl ether (3: 1). The precipitate was dissolved in 2 ml of 1 N potassium hydroxide (KOH) solution keeping in water bath at 37° C for 2 hours which hydrolyses the DNA content. DNA are again precipitated by addition of 0.4 ml of 6N Hcl and 2.6 ml of 5% TCA in a same tube. The mixture was kept at 4° C for 10 minutes and

then centrifuged. The precipitate was heated at 70° C in 2.4 ml of 5 % TCA for 15 minutes, then cooled at room temperature and centrifuged at 4000 rpm for 10 minutes. Supernatant containing DNA was collected and kept at -20° C until assay. DNA were estimated by using orcinol and diphenylamine reagent at the wave length of 660 nm and 600 nm respectively in the UV-spectrophotometer.

***Statistical analysis***

In ovary intact females the mean value of DNA of the CRE treated uterine tissue were compared with respective control of different phases of the estrous cycle. Data were analyzed using two tailed students t – test and P values < 0.05 considered as significant. In OVX females mean values of CRE treated uterus was compared with the respective control values (mean ± S.E.), i.e. water treated OVX female and sesum oil treated OVX females respectively. The data were analyzed using students t – test (paired two tails) and the P value < 0.05 was considered as significant.

## RESULTS

The DNA level in the uterine tissues remains unchanged during all phases of the estrous cycle of the ovary-intact control females (Fig. 1). A little more DNA content was observed during the estrus phase (3.19 ± 0.09 mg/ gm tissue) than the other phases. The level of DNA during proestrus (3.15 ± 0.08 mg/ gm tissue), metestrus (3.05 ± 0.10 mg/ gm tissue) and diestrus (3.01 ± 0.17 mg/ gm tissue) remained approximately unaltered. Oral administration of the CRE of *Polygonum hydropiper* increases the DNA content in all four phases of the estrous cycle in ovary-intact female (Fig. 1). A little bit of higher value was observed during the diestrus (3.18 ± 0.05 mg/ gm tissue) than the proestrus (3.18 ± 0.06 mg/ gm tissue), estrus phase (3.27 ± 0.05 mg/ gm tissue) and metestrus (3.14 ± 0.02 mg/ gm tissue) of the treated females. It was observed that the DNA content was significantly increased (P < 0.05) in the treated females following the administration of CRE than that of the control.

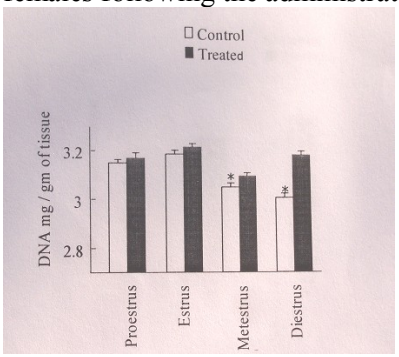


**Figure 1.** DNA content in uterine tissue of control and Polygonum hydropiper root extract treated ovary intact females during different phases of estrous cycle (values are mean ± S.E.). Oral administration of the crude root extract of Polygonum hydropiper significantly (P < 0.05) increased the DNA content in uterus tissue of female albino rat.

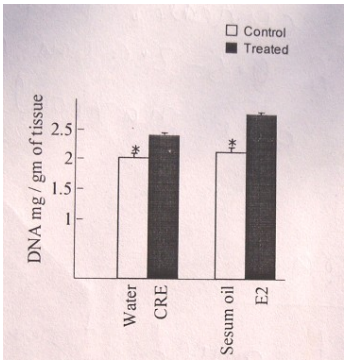


**Figure 2.** DNA contents in uterine tissue of ovariectomized (DNA) females. OVX females were treated with CRE of Polygonum hydropiper and reference drug Estradiol - 17β (E2). The control group were treated with water (vehicle for CRE) and sesum oil (vehicle for E2) in a similar manner to that of CRe and E2 respectively. Values are mean ± S. E. oral administration of CRE and S.C. injection of E2 increased the total DNA in uterus significantly.

Fig. 2 showed that the level of DNA in per gm uterine tissue in water treated OVX (control) females was (0.74 ± 0.03 mg/ gm tissue) and in CRE treated rats was 0.41± 0.02 mg/ gm tissue respectively. Subcutaneous (sc) injection of E2 decreased the value of DNA to a level of 0.41± 0.01 mg/ gm tissue. It was observed that SC administration of sesum oil increased the DNA to a maximum of 0.84 ± 0.04 mg/ gm tissue in OVX control females. Statistical analysis revealed that administration of crude root extract

and subcutaneous injection of E2 to OVX females significantly ($P < 0.05$) decreased the DNA in uterine tissues (Fig.2) than that of the respective control values.

## DISCUSSION

In the present investigation, total DNA content was quantitated in both ovary intact and OVX females. The observation showed that the total DNA content of control ovary intact females and CRE treated (ovary intact) females were significantly different. A much increased value (of the total DNA) was observed in the CRE treated group than that of the control, which was statistically significant. The results suggest that the CRE stimulate the cellular proliferation in uterine tissue; rather did not hinder the uterine cellular proliferation during all phases of estrous cycle. The reason behind the increased total DNA content in the CRE treated group may be due to the compound(s) present in the CRE. These compounds may either be having the property of phytoestrogen or other else. It is speculated in the present investigation that the various compounds present in the CRE of *Polygonum hydropiper* exert a composite effect with estrogenic property in the female reproductive organ which induces biochemical changes leading infertility.

The data of the OVX females showed that DNA level in the uterine tissues gradually decreased. In ewes, ovariectomy resulted in gradual decrease of DNA content. Following two weeks of ovariectomized, the DNA level decreased by 33% which decreased further with time (Little et.al, 1976). The effect of herbal extract on liver and uterine tissue DNA content was reported earlier (Dinman et.al. 1962 & Solomon et.al. 1993). Administration of phytoestrogen coumestrol to OVX female rats lead to increase in both dry and wet uterine weight while uterine DNA remain constant (Markaverich et. al. 1995). In addition, coumestrol treated females uterine endometrium developed hyperplasia (Ashby et.al. 1999). It is to be mentioned here that administration of synthetic estradiol – 17 β for a period of 72 hours increased DNA content in the OVX female uterine tissue. The difference between the OVX – $E_2$ treated DNA content and the OVX – vehicle treated (sesum oil) treated values were significantly different. It is clear that $E_2$ and CRE of *Polygonum hydropiper* exerts similar effect in uterine tissue of female albino rat i. e. CRE of *Polygonum hydropiper* treated as a steroidogenic compounds. The present study showed that the CRE of root of *Polygonum hydropiper* has a steroidogenic effect in mobilization of DNA in rat uterine tissue.

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