Effect of *Pueraria tuberosa* on female reproductive organs of albino rats

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Abstract- *Pueraria tuberosa* (Roxb ex Willd), Family – Fabaceae is popularly known as *Vidarikand*, English - *Kudzu*, a perennial herb found mostly in high Himalayas. It has many therapeutic properties including anti-fertility (Gupta *et al.*, 2005) in male albino rats, but, there is no mention about effect on female animals. Effect of crude root powder (*kand*) of this plant is reported in this paper by authors. Histological studies of the ovary and uterus were undertaken after oral administration of aqueous solution of the *kand* (25, 50 and 75 mg/kg/day/rat) for 30 days. The naked eye observation revealed no change and when examined histologically, there was an evidence of follicular atresia with absence of primary and secondary follicles at 50 and 75 mg kg dose for 30 days. There was no severe regressive change observed in the histoarchitecture of uterus. It appears that the plant may be used in female fertility regulation.

Keywords: Herbal drugs, Phytomedicine, Female reproduction, Contraception, *Pueraria tuberosa* D.C.

Introduction

Pueraria tuberosa D.C., family – Fabaceae (Hindi - Vidarikand; English - Kudzu) is a perennial herbaceous twinner plant found throughout India, Nepal and Pakistan and also in other Asian countries including China. At Himalayan mountain ranges. It is found upto the height of 4000 feet. It has big sized tuberous roots often rounded in shape and slightly sweet in taste. It is highly nutritious. According to Ayurveda, the tubers are aphrodisiac, diuretic and galactogogue (Kirikar and Basu, 1935). Modern researchers have reported its effects on many health problems namely anticonvulsant (Basavaraj *et al.*, 2011), antidiabetic, (Oza and Kulkarni, 2018a), anti-inflammatory (Tripathi *et al.*, 2013), antistress (Verma *et al.*, 2012), cardioprotective (Patel *et al.*, 2018), hypolipidemic (Tanwar *et al.*, 2008), immunomodulatory (Patel *et al.*, 2016), neuroprotective (Xing *et al.*, 2011),

wound healing (Kambhoja and Murthy, 2007). Concerning with reproduction, antifertility effect of this plant was reported by Gupta *et al.*, (2005) in male albino rats. Considering the above point, the effect of tubers of *Pueraria tuberosa* was carried out on female reproductive organs of albino rats (*Rattus rattus norvegicus*). The results are reported in this paper.

Phytochemistry

In the crude tuber extracts of *P. tuberosa* the following chemicals-alkaloids, anthracene, anthracyanine, anthraquinone, glycosides, carbohydrates, catecholic compounds, coumarines, flavonoides, sugars and volatile oils have been reported. Viji and Paulsamy (2015, 2018) reported above mentioned compounds including phenols and tannins using HPTLC. Pandey and Tripathi (2010) reported tuberocin, puerarostan, β-sitosterol from ethanolic tuber extract. Liquid chromatography – mass spectrometry (LC-MS) analysis of ethanolic extract was found to contain puerarin, diadzein and formononetin (Chauhan *et al.*, 2013). Two most active (pharmacologically as well as therapu-etically) chemical compounds were reported, first, Puerarone (Fig. 1a) by Maji *et al.* (2014) and second, tuberostan (Fig. 1b). These compounds are flavonoids and extracted from aqueous tuber decoction of *P. tuberose (Fig.-2)*.

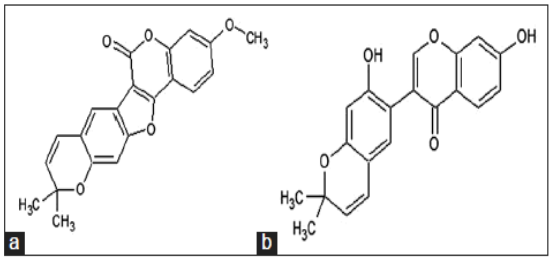

Figure- 1 Chemical structures of potential phytochemicals from *Pueraria tuberosa*: (a) Puerarone; (b) Tuberostan

Figure- 2 Tuberous root of *Pueraria tuberosa* (Roxb ex Willd) D.C.

Material and Methods

The tuber roots of *Pueraria tuberosa* D.C. were collected from Chakrata tehsil of Dehradun district of Uttarakhand in and around forested area of Tiuni. The tubers (roots) were identified by Botanists of Botanical Survey of India (B.S.I.), Northern circle, Kalagarh Road, Dehradun, Uttarakhand (India). Freshly collected air dried and mechanically powdered tuberous (tuber powder) was used as a drug. The doses 25, 50 and 75 mg/kg work prepared by adding gum acacia powder (05 mg/dose) as vehicle. Each dose was dissolved in distilled water in such a way that the dose was equivalent to 2 ml of drug solution and administered orally daily with the help of catheter tube fitted into a specially designed syringe-needle for 30 days. Control female rats (05-rats)

received vehicle only for experimental period.

Regularly cyclic adult female rats (100-120 gms body weight) were selected for the experiment. They were maintained under uniform husbandry conditions with free access of food (Hindustan Lever Limited) and tap water ad libitum. The three doses 25, 50 and 75 mg/kg were given (as described above) to group II, III and IV of rats respectively for 30 days. 05 rats were included in each group. Female rats of control group (group I) received vehicle (gum acacia powder) in the similar manner.

Albino rats were maintained as per the protocol outlined in publications of the committee for the purpose of control and supervision of experiments on animals. Standard guidelines and approval was obtained from Institutional Animal Ethical Committee appointed by the Principal for laboratory animals.

All the female rats in each group, 24 hours after the last day's dose, were sacrificed by the decapitation. The ovaries and uterii were dissected out, freed from surrounding tissues, bloated on filter paper and weighed quickly on semi-micro balance. For histological studies, ovaries and uterii were fixed in Bouin's fluid, dehydrated, paraffin embedded tissue sections were cut at 6 micron and stained with Ehrlich's Haemotoxylene and Eosine. Prepared slides of ovary and uterus were photographed and histopathological changes were described.

The body weight and organ weight prior to start of experiment and last day of experiment (30th day) was taken and recorded. The significance of difference of weight between treated and control rats was assessed by the student 't' test taking $p < 0.05$ as significant.

Results

Body and Organ Weight Changes

The results are presented in table-1. The female rats of control group did not show any change i.e. reduction in the body weight. It was maintain throughout the experimental period. Similarly, no significant reduction in body weight was noted at any dose level of *Pueraria* tuberosa, tubers powder dissolved in distilled water as solution administered for 30 days. However, the ovarian weight was reduced significantly ($p < 0.05$) after the treatment with a doses 50 and 75 mg/kg/day for 30 days. No significant reduction of weight in uterus was found at the given dose level.

Histopathological Changes In Reproductive Organs

Control: The histoarchitecture of ovaries of control rats revealed the normal cellular structures with organised germinal epithelium, all types of follicles, developing, maturing and fully mature or gravid follicles with antrum and ovum, a few atretic follicles, interstitial cells, normal vascularity and loose stroma (Figure-1). The histoarchitecture of uterii of control group of rats presented the normal features. The endometrium which surround the lumen made up of columnar epithelial cells. Lumen was wide, uterine glands were normal, tortuous and distributed in the stroma of myometrium. The musculature (myometrium) was well developed and vascularity appeared normal (Figure- 4).

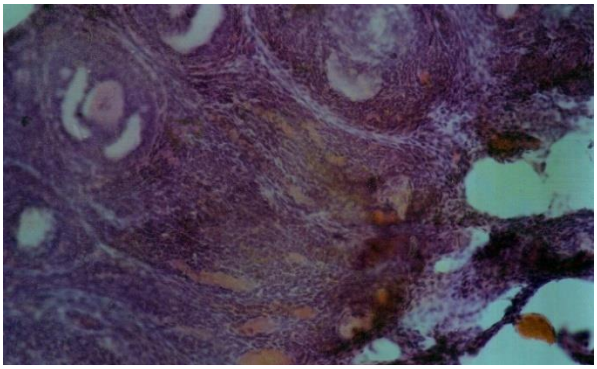

Figure-1 T.S. of ovary of control albino rats showing normal histoarchitecture with Primary (Primordial), secondary (developing) and mature (Graffian) follicles. A few atretic (degenerated) follicles, stroma and vascularity appears normal. Corpora lutea are also seen. X

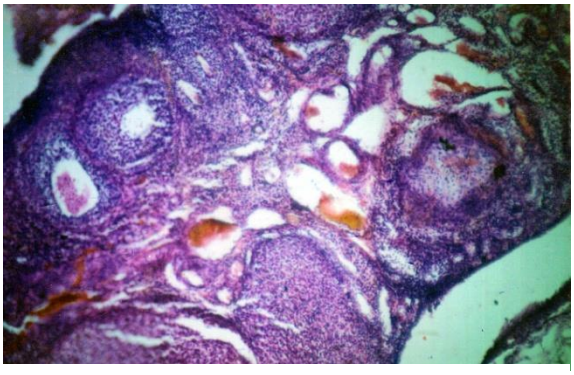

Figure- 2 T.S. of ovary of treated albino rats with *Pueraria tuberosa*, tuber aqueous solution at dose of 50 mg/kg for 30 days showing follicular atresia of primary and secondary follicles, absence of corpora lutea in loose stroma and normal vascularity. X 150

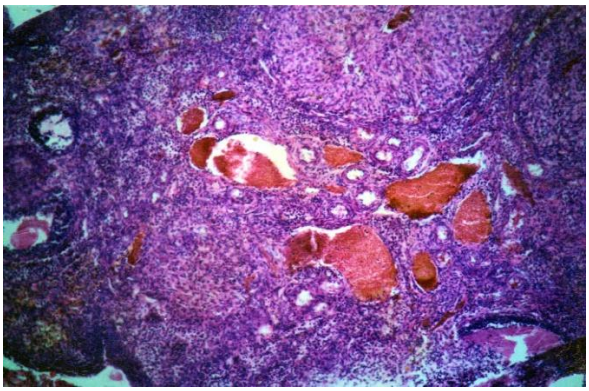

Figure-3 T.S. of ovary of treated albino rats with *Pueraria tuberosa*, tuber aqueous solution at dose of 75 mg/kg for 30 days showing mass atrophy of follicles, absence of corpora lutea compact stroma and less vascularity. X 150

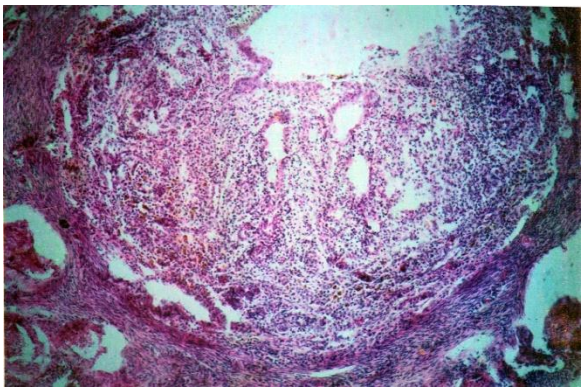

Fig.4. T.S. of uterus of control albino rats showing normal structural features. The endometrium showing columnar epithelial cells, tortuous uterine glands, normal musculature, loose stroma, wide uterine lumen and normal vascularity.. X

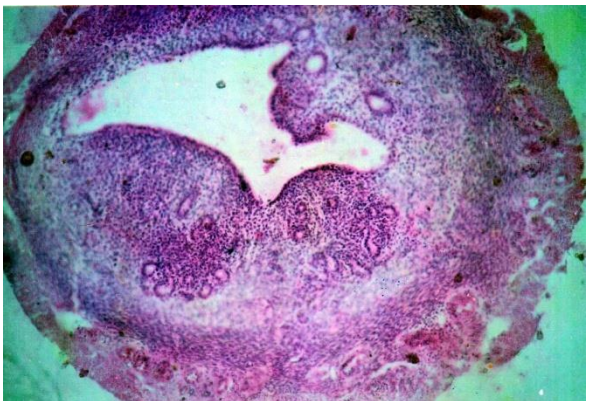

Figure-5 T.S. of uterus of treated albino rats with *Pueraria tuberosa*, tuber aqueous solution for 30 days at dose of 50 mg/kg showing less histopathological changes in the uterine elements like endometrium, uterine glands and uterine lumen. X 150

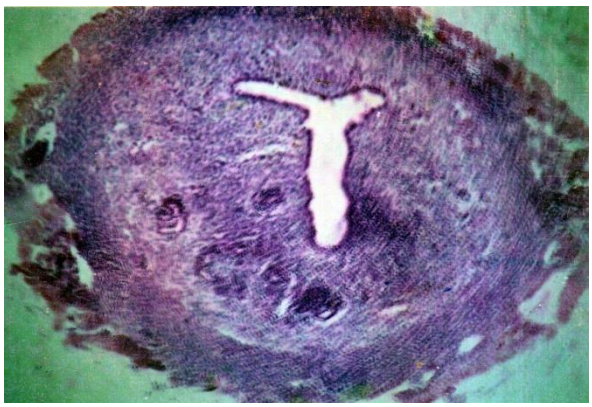

Figure-6 T.S. of uterus of treated albino rats with *Pueraria tuberosa*, tuber aqueous solution for 30 days showing mild histopathological changes in the uterine elements like distorted endometrium, shrunken uterine glands, reduced uterine lumen, stroma and vascularity. X 150

Treated (by *Pueraria tuberosa*)

The administration of 25 mg/kg/day dose of *P. tuberosa* aqueous solution for 30 days caused no histopathological changes in the ovaries. Many developing follicles can be seen with a few atretic follicles. The ovaries of rats treated with higher doses (50 and 75 mg/kg/day) for 30 days showed marked follicular atresia (degeneration of follicles) of primary and secondary follicles. Graffian (Gravid) follicles were few and appeared not much affected (Fig.2) but highly affected in the ovaries (Figure-3). There were no corpora lutea in both the cases. Primordial oocyte population was significantly reduced and degenerated. The vascularity, stromas were not much affected and appeared normal.

The administration of 25 mg/kg/day dose of *P. tuberosa* aqueous solution for 30 days did not cause histopathological changes in the uterii of treated rats. It did not differ much from the picture of uterus of control group of rats. Similarly, the doses 50 mg/kg and 75 mg/kg/day for 30 days caused mild regressive changes in the uterine elements. The endometrium, the lumen which is fully distorted appeared normal. The uterine glands were slightly reduced and not much regressed. The musculature (myometrium) and vascularity were also not much affected (Figure- 5,6).

Table-1 Effect of *Pueraria tuberosa* tuber powder as aqueous solution on body weight (gms) and genital organ weight (milli gms) of female rats treated with various doses for 30 days. 05 rats were used in each group. Values are mean ± S.E.

Doses (mg/kg)	Body weight (gms)		Genital organ weight (milli gms)	
	Initial	Final	Ovaries	Uterii
Control	110.30 ± 06.40	130.15 ± 11.20	84.27 ± 17.92	110.25 ± 10.35
25	105.20 07.50	102.10 ± 07.15	80.25 ± 13.98	108.17 ± 25.90
50	112.52 ± 17.20	110.13 ± 09.12	53.15 ± 17.92*	95.25 ± 15.13
75	115.10 ± 10.23	112.17 ± 10.15	50.20 ± 25.65*	90.14 ± 37.52

* $p < 0.05$

Discussion

In the present study which deals with effect of *Pueraria tuberosa* D.C. tuber root crude powder as aqueous solution/ suspension on reproductive organs i.e. ovaries and uteri of female albino rats. According to studies, the ovarian and uterine weight was reduced by administration of the above plant with the increasing dose level 25 mg, 50 mg and 75 mg/kg/day ($p < 0.05$). The doses 50 and 75 mg/kg caused deleterious/regressive effect on ovarian elements such as primordial, developing, mature and Graffian follicles. The doses increased formation of atretic follicles. i.e. follicular atresia.

The formation of follicles with normal ova, ovulation. fertilization, implantation and change in the estrous cycle and genital organ weight (ovary and uterus) are controlled by hormones, oestrogen and progesterone (Lerner, 1969). Pincus *et al.* (1956) reported that the reduction of ovarian weight was due to suppression of endogenous oestrogen or progesterogenic action. Pandey (1990) reported that the administration of *Adhatoda vasica* leaves as aqueous extract caused widespread

damage to all ovarian elements including reduction in weight. Singh (1017) observed atrophic changes in ovary and uterus caused due to administration of *Stevia rebaudiana* leaves. Present study of histopathological changes in female reproductive organs are comparable to the studies made by Chakraborti *et al.* (1968) when female albino rats fed with green leaves of *Artobotrys odoratissimus* Linn. The follicular atresia and other degenerative changes in ovary which have similarity with present observations. The results reported in the present study on *Pueraria tuberosa* are similar with results reported by Dixit (1977) by administration of *Malva viscus conzattii* flower extract in female genital organs of Indian gerbil (*Meriones hurrianae* Jerdon). Singh and Singh (1992) also reported similar histopathological in the ovary and uterus after administration of *Cassia fistula* flower extract in female albino rats within 30 days at different doses. The dose 25 mg/kg dose for 30 days did not cause histopathological and other changes in reproductive organs of female albino rats but 50 and 75 mg/kg doses caused deleterous/ regressive changes in the present study.

Conclusion

As stated above *Pueraria tuberosa* D.C. is very popular for traditional remedy among people of India and China including other Asian countries. Its tubers and leaves are used as folk medicine. It has shown medicinal value for anticancer, anticonvulsant, antidiabetic, anti-inflammatory, antioxidant, antistress, cardioprotective, hepatoprotective, immu-nemodulatory, neuroprotective and wound healing. Antifertility activity in male albino rats was already reported and effect of tubers on female reproductive organs i.e. ovaries and uterii are reported in this paper. It has two very important and active phytochemical, besides many others, known as flavonoides – Puerarone and Tuberostan. According to the present study, the tubers of *P. tuberosa* have shown regressive deleterious effect on ovary and uterus of female albino rats. It is concluded that the plant material – tubers of *Pueraria tuberosa* may be useful to control female reproductive function for family planning.

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Disclaimer Statment

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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